DEVELOPMENT OF ANALYTICAL METHODS FOR THE ESTIMATION OF TORSEMIDE AND SPIRONOLACTONE IN TABLET DOSAGE FORM

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MASTER OF PHARMACY

(Pharmaceutical Analysis)

Submitted by

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CERTIFICATE

This is to certify that the research work entitled **DEVELOPMENT OF ANALYTICAL METHODS FOR THE ESTIMATION OF TORSEMIDE AND SPIRONOLACTONE IN TABLET DOSAGE FORM** submitted to The Tamil Nadu Dr.M.G.R. Medical University in partial fulfillment for the award of the Degree of Master of Pharmacy (Pharmaceutical Analysis) was carried out by **K.VARADHARAJAN (Reg.No.26083279)** in the Department of Pharmaceutical Analysis under my direct guidance and supervision during the academic year 2009–2010.

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This is to certify that the dissertation entitled **DEVELOPMENT OF ANALYTICAL METHODS FOR THE ESTIMATION OF TORSEMIDE AND SPIRONOLACTONE IN TABLET DOSAGE FORM** is the bonafide research work carried out by **K.VARADHARAJAN (Reg.No.26083279)** in the Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr.M.G.R. Medical University under the guidance of Prof. (Dr.) T.VETRICHELVAN, M.Pharm., Ph.D.,

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LIST OF ABBREVIATIONS USED

% RSD	-	Percentage Relative Standard Deviation
% v/v	-	Percentage volume/volume
%	-	Percentage
λ	-	Lambda
µg	-	Microgram
λ_{max}	-	Absorption maximum
µ	-	Micron
AUC	-	Area under the curve
HCl	-	Hydrochloric Acid
HPLC	-	High Performance Liquid Chromatography
IP	-	Indian Pharmacopoeia
LOD	-	Limit of Detection
LOQ	-	Limit of Quantitation
mg	-	Milligram
ml	-	Milliliter
mM	-	Millimole
ng	-	Nanogram
nm	-	Nanometer
r	-	Regression coefficient
RP-HPLC	-	Reverse Phase High Performance Liquid Chromatography
S.D	-	Standard Deviation
S.E	-	Standard Error
USP	-	United States Pharmacopoeia
UV	-	Ultraviolet
MCG	-	Microgram

DEDICATED

PARENTS

Introduction

1. INTRODUCTION

1.1 ANALYTICAL METHODS (Alexeyev, 2004)

Analytical chemistry is a science concerned with development of the theoretical foundations and methods of chemical analysis. The practical objective of analytical chemistry is to establish the chemical composition of substances or their mixtures.

The task of analytical chemistry can be solved with the aid of a variety of methods, either by chemical, physical, physio-chemical methods. These methods are continuously expanded and perfected. More advanced techniques of analysis are continuously designed, which require new procedures and new theoretical substantiation. Analytical chemistry can be divided into two areas called qualitative analysis and quantitative analysis. The detection or identification of individual of elements or ions entering into the composition of a substance constitutes the task of qualitative analysis. The determination of the quantitative content of constituents of the substance under study is the aim of quantitative analysis.

1.1.1 Classification of Analytical Methods (Gary D. Christian, 2005)

Many available techniques possess varying degrees of selectivity, sensitivity, accuracy & precision, cost and rapid.

1. Gravimetric Analysis
2. Volumetric / Titrimetric analysis
3. Instrumental analysis

A) Spectrophotometry
   1. Ultraviolet spectroscopy
   2. Visible Spectrophotometry
   3. IR spectroscopy

B) Fluorimetry

C) Atomic Spectroscopy
   1. Absorption
   2. Emission

D) Nuclear Magnetic Resonance Spectroscopy(NMR)

E) X-ray spectroscopy
    1. Absorption
    2. Fluorescence
F) Electro analytical chemistry
    1. Potentiometry
    2. Voltammetry
G) Chromatography
    1. Gas
    2. Liquid
H) Radio chemistry

1.1.2 Stages of Analysis (Mendham *et al*., 2002)

A complete chemical analysis, even for a single substance, involves a series of steps and procedures. These steps assessed in order to minimize errors and to maintain accuracy and reproducibility.

The steps are

1. Sampling
2. Preparation of Analytical sample
3. Dissolution of sample
4. Removal of interference
5. Sample measurement and control of Instrumental factors.
6. Results
7. Presentation of data.

1.1.2 Factors Affecting the Choice of Analytical Method (Mendham *et al*., 2002)

Analytical techniques have different degrees of sophistication, sensitivity and selectivity as well as different cost and time requirements. An important task for the analyst is to select the best procedure for a given determination. This will require careful consideration of the following criteria.

1. The type of analysis required elemental or molecular, routine or occasional.
2. Problems arising from the nature of the material to be investigated.
3. The concentration range to be investigated
4. The accuracy required.
5. The facilities available, particularly the instruments.

1.2 ULTRAVIOLET SPECTROSCOPY

Introduction (Kalsi, 2007; Sharma, Y.R. 2007; Fifield *et al.,* 2004)

Ultraviolet spectroscopy deals with the measurement of energy absorbed when electrons are promoted to higher energy levels. On passing electromagnetic radiation in the ultraviolet and visible regions through a compound with multiple bonds, a portion of the radiation is normally absorbed by the compound. The amount of absorption depends on the wavelength of the radiation and the structure of the compound. Absorption of electromagnetic radiation in the visible and ultraviolet regions of the spectrum results in changes on the electronic structure of ions and molecules.

Ultraviolet spectrum records the wavelength of an absorption maximum, i.e. λ_{max} and the strength of the absorption, i.e. molar absorptivity (extinction co-efficient Σ_{max}) as defined by the combined Beer-Lambert's law.

The ultraviolet spectrum will be divided into the following region.

i) Far (or vacuum) ultra violet → 10 – 200 nm

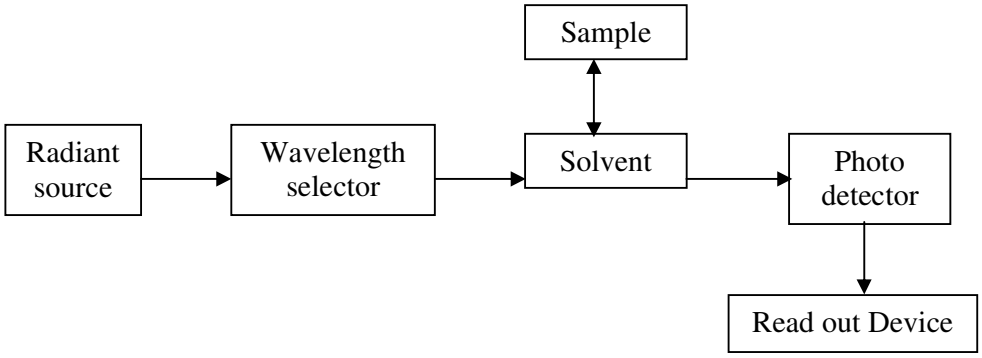
ii) Near or quartz ultraviolet → 200 – 380 nm

iii) Visible region → 380 – 780 nm

The visible region corresponds to 400-800 nm and ultraviolet region to 200-400 nm.

Instrumental modules for measuring absorption of UV radiation

(Willard *et al*., 1986)



Absorption Laws (Jag Mohan, 2006)

The absorption of light by the molecules is governed by the following laws.

a) Beer's law

It states that the fraction of the incident light absorbed is proportional to the number of the absorbing molecules in the light path and will increase concentration or sample thickness.

b) Lambert's law

It states that the fraction of the monochromatic light absorbed by a homogeneous medium is independent of the intensity of the incident light and each successive unit layer absorbs an equal fraction of the light incident on it.

From the laws, the following empirical expression, known as Beer – Lambert's law

$$\text{Log}_{10}\left(\frac{I_0}{I}\right) = \sum cl = A$$

Where,	I_o	-	Intensity of incident light
	I	-	Intensity of emerged light
	Σ	-	Molar absorptivity
	c	-	Concentration of solute in moles/litre
	l	-	Path length (Cm)
	A	-	Absorbance

Limitations of Beer-Lambert's Law

The Beer-Lambert law is rigorously obsessed provided a single species gives rise to the observed absorption. However the law may not obsessed when,

i) Different forms of the absorbing molecules are in equilibrium.

ii) Solute and solvent from association complexes.

iii) There is a thermal equilibrium between ground electronic state and a low lying excited state.

iv) The compounds are charged by irradiation (fluorescent compounds).

Deviations from Beer's Law (Sharma, B.K, 2000)

According to Beer's law, a straight line passing through the origin should be obtained, when a graph is plotted between absorbance (A) and concentration. Deviation from the law may be positive or negative, according to whether the resulting curve is concave upwards or concave downwards.

The deviations from the Beer's law may be due to interaction of the solute molecules with each other or with the solvent or may be due to instrumental factors. The most important reasons that cause deviations are

i) Negative deviation can always be expected when the illumination is not monochromatic.

ii) The presence of impurities that fluoresces or absorb at the required absorption wavelength.

iii) Environmental errors such as solvent, temperature and pressure.

iv) Chemical factors such as change in pH and chemical equilibrium, presence of complexing agent, competitive metal ion reactions and concentration dependence.

v) Refractive index of sample.

vi) Instrumental errors such as radiation, stability of radiation source, stability of slit control and electronics and reliability of the optical parts.

Choice of solvent (Chatwal, 2000)

A suitable solvent for ultraviolet spectroscopy should meet the following requirements.

a) It should not itself absorb radiations in the region under investigation.

b) It should be less polar so that it has minimum interaction with the solute molecules.

c) The solvent used should be of high purity.

Following table gives a list of common solvents used in UV spectroscopy (Williams, 2007)

	Solvents	**Minimum wavelength for 1cm cell (nm)**
1	Acetonitrile	190
2	Carbon tetrachloride	257
3	Chloroform	237
4	Cyclohexane	195
5	Ether	215
6	Ethanol	204
7	Hexane	201
8	Methanol	203
9	Methylene dichloride	220
10	Water	191

Solvent Effects (William Kemp, 1996)

The position and intensity of an absorption band may shift when the spectrum is recorded in different solvents. For changes to solvents of increased polarity we can summarize the normal pattern of shifts as follows.

1. Conjugated dienes and aromatic hydrocarbons experience very little solvent shift.
2. α,β unsaturated carbonyl compounds show two different shifts.
   i) The $\pi - \pi^*$ bond moves to longer wave length (red shift)
   ii) The $n - \pi^*$ bond moves to shorter wavelength (blue shift)

Wavelength Choice (Robert D Braun, 2006)

When choosing a wavelength at which to make an absorbance measurement, three factors must be considered.

Factor 1

If the solution contains more than one absorbing species, the wavelength that should be choosen, whenever possible, is that at which the second species in the solution does not absorb radiation.

Factor 2

It must be considered when a wave length is choosen is the required sensitive of the assay.

Factor 3

This factor to be considered is the sensitive of the assay to small changes in wavelength it is preferable to choose a wavelength at which the absorbance will not be significantly altered if the wavelength is slightly changed.

Detectors (Sharma, B K, 2000; Willard *et al*., 1986)

A detector is a transducer that convert electromagnetic radiation into an electron flow and subsequently, into a current flow or voltage in the readout circuit. Photoelectric or photo multiplier tubes are generally used as detectors.

The detector must have the following important requirements.

a) It must respond to radiant energy over a broad wavelength range.
b) It should be sensitive to low levels of radiant power.
c) It should rapidly respond to the radiation and produce an electrical signal that can be readily amplified.
d) It should have relatively low noise level (for stability)
e) The signal produced is directly proportional to the power of beam striking it.

Qualitative analysis

The assay of an absorbing substance may be quickly carried out by preparing a solution in a transparent solvent and measuring its absorbance at a suitable wavelength. The wavelength normally selected is a wavelength of maximum absorption (λ_{max}) where small errors in setting the wavelength scale have little effects on the measured absorbance.

Quantitative analysis (Beckett and Stenlake, 2002)

Assay of Substances in single Component Samples

Absorption spectroscopy is one of the most useful tools available to the chemist for quantitative analysis. The most important characteristics of photometric and spectrophotometric methods are high selectivity and ease of convenience. Quantitative analysis (assay of an absorbing substance) can be done using following methods.

- Use of A (1%, 1cm) values
- Use of calibration graph (multiple standard methods)
- By single or double point standardization method

Use of A (1%, 1cm) values

This method can be used for the estimation from formulation or raw material when reference standard not available. The use of standard A (1%, 1cm) value avoids the need to prepare a standard solution of the reference substance in order to determine its absorption.

Use of calibration graph

In this method a calibration curve is plotted using concentration (X-axis) Vs absorbance (Y-axis) with the value of 5 or more standard solutions. A straight line is drawn through maximum number of points. This method is called as line of best fit. By interpolating the absorbance of the sample solution and using the calibration curve, the concentration of the drug amount and percentage purity can be calculated.

Single or double point standardization

The procedure involves the measurement of the absorbance of a sample solution and of a standard of the reference substance. The standard and the sample solution are prepared in similar manner; ideally the concentration of the standard solution should be close to that of the sample solution. The concentration of the substance in the sample is calculated using following formulas,

$C_{test} = A_{test} / A_{std} \times C_{std}$

Where,

C_{test} and C_{std} are the concentration of the sample and standard solutions, respectively.

A_{test} and A_{std} are the absorbance of the sample and standard solutions, respectively.

In double point standardization, the concentration of one of the standard solution is greater than that of the sample while the other standard solution has a lower concentration than the sample solution. The concentration of the substance in the sample solution is given by

$$C_{test} = \frac{(A_{test} - A_{std1})(C_{std1}-C_{std2}) + C_{std1}\,(A_{std1}-A_{std2})}{A_{std1}-A_{std2}}$$

Where,

C_{std} is the concentration of the standard solution.

A_{test} and A_{std} are the absorbance of the sample and standard solutions, respectively.

Std 1 and Std 2 are the more concentrated standard and less concentrated standard solutions, respectively.

Assay of Substances in Multi Component Samples

The spectrophotometry assay of drugs rarely involves the measurement of absorbance of samples containing only one absorbing component. The pharmaceutical analyst frequently encounters the situation where the concentration of one or more substances is required in samples known to contain other absorbing substances which potentially interfere in the assay. Unwanted absorption from these sources is termed irrelevant absorption and if not removed, imparts systematic errors to the assay of the drug in the sample. A number of modifications to the simple spectrophotometric procedure for single-component samples are available to the analyst, which may eliminate certain sources of interferences and permit the accurate determination of one or all of the absorbing components.

The basis of all the spectrophotometric technique for multi-component samples is the property that at all wavelengths:

a) The absorbance of a solution is the sum of absorbance of the individual components.

b) The measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell.

The determination of the multi-component samples can be done by using the following methods.

- Assay of a single-component sample
- Assay using Absorbance corrected for interference
- Assay after solvent extraction of the sample
- Simultaneous equation method
- Absorbance ratio method (Q-Analysis)
- Geometric correction method
- Orthogonal Polynomial Method
- Difference Spectrophotometry
- Derivative Spectrophotometry and
- Chemical derivatisation

Simultaneous equation method

If a sample contains two absorbing drugs (X and Y) each of this absorbs at the λmax of the other. It may be possible to determine both drugs by the technique of simultaneous equation (Vierodt's method).

The Informations required are as follows,

a) The absorptivities of X at λ_1 and λ_2, ax_1 and ax_2, respectively
b) The absorptivities of Y at λ_1 and λ_2, ay_1 and ay_2, respectively
c) The absorbance of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.

Let Cx and Cy, be the concentrations of X and Y respectively in the diluted sample.

Two equations are constructed based upon the fact that at λ_1 and λ_2, the absorbance of the mixture is the sum of the individual absorbances of x and y,

At λ_1 $A_1 = ax_1bcx + ay_1bcy$ ---------- (1)

At λ_2 $A_2 = ax_2bcx + ay_2bcy$ ---------- (2)

For measurements in 1 cm cells, b=1. Rearrange eq. (2)

$$c_y = \frac{A_2 - a_{x_2} c_x}{a_{y_2}}$$

Substituting for c_y in eq. (1) and rearranging gives

$$c_x = \frac{A_2 a_{y_1} - A_1 a_{y_2}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \quad (3)$$

$$c_y = \frac{A_2 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \quad (4)$$

Absorbance ratio method

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that, for a substance which obeys Beer's law at all wavelengths, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length. For example, two different dilutions of the same substance give the same absorbance ratio A_1/A_2, 2.0. In the USP, this ratio is referred to as a Q value. The British Pharmacopoeia also uses a ratio of absorbance at specified wavelengths in certain confirmatory tests of identity. For example, Cyanocobalamine exhibits three λ_{max} at 278 nm, 361 nm and 550 nm. The A_{360}/A_{550} is required to be 3.30 ± 0.15 and the A_{361}/A_{278} to be 1.79 ± 0.09.

Geometric correction method

A number of mathematical correction procedures have been developed which reduce or eliminate the background irrelevant absorption that may be present in samples of biological origin. The simplest of these procedures is the three-point geometric procedure, which may be applied if the irrelevant absorption is linear at the three wavelengths selected.

Orthogonal polynomial method

The technique of orthogonal polynomials (Glenn, 1963) is another mathematical correction procedure which involves more complex calculations than the three –point correction procedure. The basis of the method is that an absorption spectrum may be represented in terms of orthogonal functions as follows:

$A(\lambda) = p_0P_0(\lambda) + p_1P_1(\lambda) + p_2P_2(\lambda) \ldots p_nP_n(\lambda)$

Where A denotes the absorbance at wavelength λ belonging to a set of n + 1 equally spaced wavelengths at which the orthogonal polynomials, $P_0(\lambda)$, $P_1(\lambda)$, $P_2(\lambda) \ldots P_n(\lambda)$ are each defined.

Area under the curve method

From the spectra obtained for calculating the simultaneous equation, the area under the curve were selected at a particular wavelength range for both the drugs were each drug have its absorption. The "X" values of the drugs were determined at the selected AUC range. The "X" value is the ratio of area under the curve at the selected wavelength range with the concentration of the component in mg/ml. These "X" values were the mean of six independent determinations. A set of two simultaneous equations were obtained by using mean "X" values. And further calculations are carried out to obtain the concentration of each drug present in the sample.

Derivative spectrophotometry

Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. The transformations that occur in the derivative spectra are understood by reference to a Gaussian band which represents an ideal absorption band. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order or D° spectrum. The first derivative (D^1) spectrum is a plot of the ratio of change of absorbance with wavelength against wavelength.

These spectral transformations confer two principle advantages on derivative spectrophotometry. Firstly, an even order spectrum is of narrower spectral bandwidth than its fundamental spectrum. A derivative spectrum therefore shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of the λ_{max} of the individual bands. Secondly, derivative spectrophotometry discriminates in favour of substances of narrow spectral bandwidth against broad bandwidth substances. This is because 'the derivative amplitude (D), i.e. the distance from a maximum to a minimum, is inversely proportional to the fundamental spectral bandwidth (W) raised to the power (n) of the derivative order.

Thus, $\mathbf{D \alpha (1/W)^n}$

All the amplitudes in the derivative spectrum are proportional to the concentration of the analyte, provided that Beer's Law is obeyed by the fundamental spectrum. The measured value in a quantitative assay is the largest amplitude that is unaffected by the presence of other, absorbing components of the sample.

Difference spectrophotometry

The selectivity, accuracy of spectrophotometric analysis of samples containing absorbing interferences may be markedly improved by the technique of difference spectrophotometry. The essential future of a difference spectrophotometric assay is that the measured value is the difference absorbance (A) between two equimolar solutions of the analyte in different chemical forms which exhibit difference spectral characteristics. The criteria for applying difference spectrophotometry to the assay of a substance in the presence of other absorbing substances are that,

- Reproducible changes may be induced in the spectrum of the analyte by the addition of one or more reagents.
- The absorbance of the interfering substances is not altered by the reagents.

The simplest and most commonly employed technique for altering the spectral properties of the analyte is the adjustment of the pH by means of aqueous solutions of acid, alkali or buffers. The ultra violet visible absorption spectra of many substances containing ionisable functional groups, e.g. phenols, aromatic carboxylic acids and amines are dependent on the state of ionization of the functional groups and consequently on the pH of the solution. The measured value in a quantitative difference spectrophotometric assay is the ΔA at any suitable wavelength measured to the baseline, e.g. ΔA_1 at λ_1 or amplitude between an adjacent maximum and minimum,

e.g. ΔA_2 at λ_2 and λ_1

$\Delta A = A_{ALK} - A_{ACID}$

Where A_{alk} and A_{acid} are the individual absorbances at λ_1 in 0.1M sodium hydroxide and 0.1M hydrochloric acid solution, respectively.

$\Delta A = \Delta abc$

1.3 CHROMATOGRAPHY (Gary D. Christian, 2005; Willard *et al*., 1986)

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase), while the other (the mobile phase) moves in a definite direction. Chromatography was invented and named by the Russian Botanist Mikhail Tswett at the beginning of the 20th Century.

1.3.1 Methods in Chromatography (Sethi, 2001; Mendham *et al*., 2002)

1. Absorption Chromatography

- Normal Phase Chromatography
- Reversed Phase Chromatography
- Ion exchange Chromatography
- Affinity Chromatography
- Hydrophobic Interaction Chromatography (HIC)

2. Partition Chromatography

- Gas Chromatography
- Liquid -Liquid Partition Chromatography

3. Size Exclusion Chromatography (SEC)

- Gel permeation Chromatography
- Gel Filtration Chromatography

Normal Phase Chromatography

The term normal phase Chromatography is used when the stationary phase is polar and the mobile phase is non-polar. In this case the solute elution order is based on the principle that non-polar solutes prefer the mobile phase and elute earlier, whereas polar solutes prefer the stationery phase and elute later.

Reverse Phase Chromatography

In Reverse phase chromatography (RPC) the stationary phase is non-polar and the mobile phase is polar, the solute elution order is commonly the reverse of that observed in normal phase chromatography, i.e. polar compounds elute earlier and non polar compounds elute later.

1.3.2 High Performance Liquid Chromatography (Francis Rouessac, 2000)

High performance liquid chromatography (HPLC) is an analytical technique in general use. It evolved from preparative column chromatography and its performance (efficiency, resolution) has been greatly enhanced by the use of elaborate stationary phases composed of spherical particles with diameter ranges between 2 and 5 µm. However, because the particles are small, the head pressure needed to force the mobile phase through the column packing must be greatly increased compared to that used in preparative liquid chromatography. Because of this, the letter P in HPLC has occasionally corresponded to the word 'pressure'.

High performance liquid chromatography (HPLC) is the most versatile and widely used type of elution chromatography. The technique is used by chemists to separate and determine species in a variety of organic, Inorganic and biological materials.

1.3.3 RP-HPLC (Mendham *et al*., 2002)

High performance systems have meant that liquid chromatography has over taken gas chromatography as HPLC now provide the following features,

1. High Resolving Power
2. Speedy separation
3. Continuous monitoring of the column effluent
4. Accurate quantitative measurement
5. Repetitive and reproducible analysis using some columns
6. Automation of the Analytical procedure and data handling

1.3.4 Mobile Phase

A successful chromatographic separation depends upon differences in the interaction of the solutes with the mobile phase and the stationary phase. In liquid chromatography, the choice and variation of the mobile phase is of critical importance in achieving optimum efficiency. HPLC grade solvents tend to be costly. To ensure consistent performance, the solvent should not contain any trace amounts of other materials, including water for organic solvents.

1.3.5 Pumping Systems (Douglas *et al*., 2006; Willard *et al*., 1986)

The requirements for liquid chromatographic pumps include

- Ability to generate pressures of up to 6000 psi (Ibs/in^2)
- Pulse free output
- Flow rate ranging from 0.1 to 10 ml/min
- Flow reproducibility of 0.5% relative or better
- Resistance of corrosion by a variety of solvents

Types of pumps

- Reciprocating Piston Pumps
- Syringe Type Pump
- Constant Pressure Pump

1.3.6 Columns for HPLC (Willard *et al*., 1986)

The columns most commonly used are made from precision bore polished stainless steel tubing; typical dimensions are 10-30 cm long and 4 (or) 5 mm internal diameter. The stationary phase (or) packing is retained at each end by thin stainless steel frits with a mesh of 2 µm or less. The packing used in modern HPLC consist of small, rigid particles having a narrow particles size distribution. The types of column used in HPLC are

- Standard columns
- Radial compression columns
- Narrow Bore columns
- Short, fast columns
- Guard columns and In-line filters

1.3.7 Temperature Control

Separation columns should be housed within a stable system with temperature variations of less than 0.1°C, the temperature changes must be avoided. Circulating air bath or electrically heated chambers are used to control the column temperature. The solvent is preheated separately before entering the separation column.

1.3.8 Detectors (Ashutoshkar, 2005)

The main function of the detector in HPLC is to monitor the mobile phase coming out the column, which in turn emits electrical signals that are directly proportional to the characteristics either of the solute or the mobile phase.

1.3.9 Applications of RP-HPLC

Chromatography has grown to be the premier method for separating closely related chemical species. In addition, it can be employed for qualitative identification and quantitative determination of separated species.

1.3.9.1 Qualitative analysis (Douglas *et al*., 2006)

A chromatogram provides only a single piece at qualitative information about each species in a sample, namely, its retention time or its position on the stationary phase after a certain elution period. Additional data can of course be derived from chromatograms involving different mobile phases and stationary phases and various elution temperatures. Still the amount of information obtainable by chromatography is small compared with the amount provided by a single IR, NMR or mass spectrum. Further more spectral wavelength or frequency data can be determined with much higher precision than can their chromatographic counter part (t_R). It is important to note that the chromatograms may not lead to positive identification of species present in a sample, but they often provide sure evidence of the absorbance of certain compounds. If the sample does not produce a peak at the same retention time as a standard run under identical conditions, it can be assumed that the compound is absent (or is present at a concentration level below the detection unit of the procedure).

1.3.9.2 Quantitative analysis (Lloyd R. Snyder *et al*., 1997)

Quantitative chromatography is based upon a comparison of either the height or the area of the analyte peak, with that of one or more standards. The different calibration methods are given as the following

Normalized Peak Area

After completion of a run and the integration of all significant peaks in the chromatogram, the total peak area can then be calculated. The area present of any individual peak is referred to as the normalized peak area.

External Standard Calibration

Determination of the concentration of an unknown sample by constructing a calibration plot using external standards. Standard solutions (some times called calibrators) are prepared at known concentrations. A fixed volume of each standard solution is injected and analyzed and the peak responses are plotted vs. concentration. The calibrators are referred to as external standard. Concentration is determined graphically from a calibration plot, or numerically using response factors.

Internal Standard Calibration

In this technique for calibration involves the addition of an internal standard to the calibration solutions and samples. The internal standard is a different compound from the analyte.

The main use of internal standard is it can compensate for changes in sample size or it can compensate for changes in concentration due to instrumental variations or certain samples require significant pretreatment, E.g. filtration, extraction etc., which results in sample loss. So the internal standard is added prior to sample preparation to correct for sample loss. A calibration plot is produced by preparing and analyzing calibration solutions containing different concentration of the internal standard added. The peak area ratio of standard and internal standard added vs. standard concentrations. The plot can be used directly to determine the concentration of sample using slope and intercept.

Method of Standard Addition

Different weights of analyte are added to the sample matrix, which initially contains an unknown concentration of the analyte. Extrapolation of a plot response found for the standard addition calibration concentrations to zero concentration defines the original concentration in the un-spiked sample.

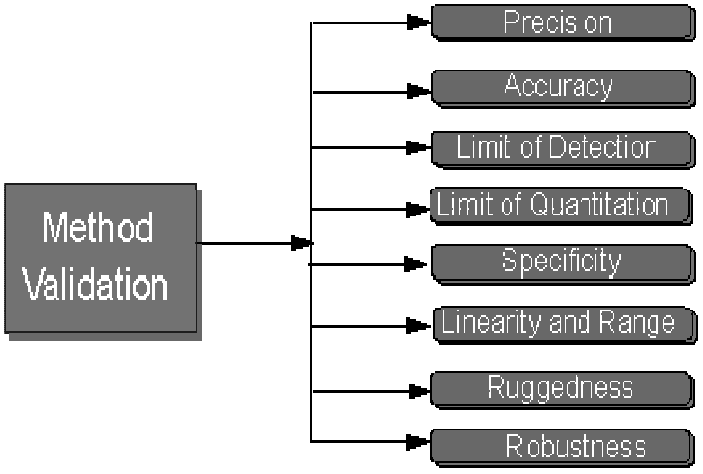
1.4 ANALYTICAL METHOD VALIDATION

(Code Q2A; Q2B, ICH Guidelines - 1994 and 1996; USP 1995)

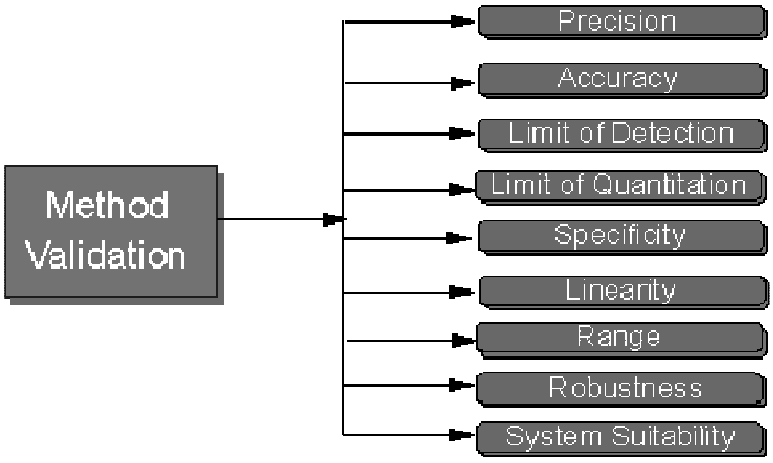
Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Methods need to be validated or revalidated,

- Before their introduction into routine use
- Whenever the conditions change for which the method has been validated e.g., instruments with different characteristics.
- Whenever the method is changed, and the change is outside the original scope of the method.
- The International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceutical for human use has developed a consensus text on the validation of analytical procedures. The document includes definitions for different validation characteristics.

VALIDATION FOR UV-VISIBLE SPECTROSCOPY METHOD- USP



VALIDATION FOR HPLC METHOD – ICH GUIDELINE



1.4.1. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.
An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and the assay. The procedures used to demonstrate specificity will depend on the intended objective of the analytical procedure.

1.4.2. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or on an accepted reference value and the value found.

1.4.2.1. Assay

- Assay of Active Substance
- Assay of Medicinal products

several methods are available to determine the accuracy.

a. Application of an analytical procedure to an analyte of known purity (e.g. reference material).
b. Comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined (independent procedure).

c. Application of the analytical procedure to synthetic mixtures of the product components to which known quantities of the substance to be analyzed have been added.

1.4.2.2. Impurity (Quantification)

Accuracy should be assessed on sample (substance /products) spiked with known amounts of impurities. It should be clear how the individual or total impurities are to be determined.

1.4.3. Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed at the variance, standard deviation or coefficient of variation of a series of measurements. Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

1.4.3.1. Repeatability

Express the precision under the same operating conditions over a short interval of time. Repeatability is also termed as intra - assay precision. It should be assessed using a minimum of nine determinations covering the specified range for the procedure (e.g. three concentration/three replicates each) or a minimum of determinations at 100% of the test concentration.

1.4.3.2. Intermediate Precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipments, etc.

1.4.3.3. Reproducibility

Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance inclusion of procedures in Pharmacopoeias.

1.4.4. Linearity

Linearity of an analytical procedure is its ability (with in a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods. For example, calculation of a regression line by the method of least square. Therefore data from regression line itself may be helpful to provide mathematical estimates of the degree of linearity.

1.4.5. Range

Range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample including these concentrations for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

The following minimum specified ranges should be considered

- For the assay of an active substance or a finished product normally from 80-120 % of the test concentration.
- For the content uniformity, covering a minimum of 70-130 % of the test concentration.
- For dissolution testing, 20% over the specified range (e.g.), If the specifications for a controlled release product cover a region from 20% after 1hour, up to 90% after 24 hours, the validated range would be 0-110% of label claim.

1.4.6. Limit of Detection

The Detection Limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.

1.4.6.1. Based on visual evaluation

Visual evaluation may be used for non-instrumental methods but may be used with instrumental methods.

1.4.6.2. Based on signal-to-noise ratio

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio 3 or 2:1 is generally considered acceptable for estimating the detection limit.

1.4.6.3. Based on the standard deviation of the response and the slope

The Detection Limit (DL) may be expressed as

$$DL = 3.3\sigma/S$$

Where,

σ = The standard deviation of the response

S = The slope of the calibration curve

The slope, S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways.

1.4.6.3.1. Based on the Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

1.4.6.3.2. Based on the calibration curve

A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the

standard deviation of y-intercept of regression lines may be used as the standard deviation.

1.4.7. Limit of Quantification

The quantification limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

1.4.7.1. Based on visual evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods.

1.4.7.2. Based on Signal- to-Noise ratio

Determination of the Signal-to-Noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical Signal- to-Noise ratio is 10:1.

1.4.7.3. Based on the Standard Deviation of the Response and the slope

The Quantification Limit (QL) may be expressed as

$$QL = \frac{10\sigma}{S}$$

Where,

σ = The standard deviation of the response

S = The slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

1.4.7.3.1. Based on standard deviation of the blank

Measurement of the magnitude of analytical background response is performed by an appropriate number of blank samples and calculating the standard deviation of these responses.

1.4.7.3.2. Based on the Calibration Curve

A specific calibration curve should be studied using samples containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

1.4.8. Robustness

The evaluation of robustness should be considered during the development phase and it depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

Incase of liquid chromatography, examples of typical variations are

- Influence of variation of pH in a mobile phase
- Influence of variations in mobile phase composition
- Different columns (different lots and / or suppliers)
- Temperature
- Flow rate

In the case of gas-chromatography, examples of typical variations are

- Different columns (different lots and / or suppliers)
- Temperature
- Flow rate

1.4.9 Ruggedness

The United States pharmacopoeia (USP) define ruggedness as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysts, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst.

1.5 SYSTEM SUITABILITY PARAMETERS

(Anonymous, USP XXIII 1995; Beckett and Stenlake, 2002; Willard *et al*., 1986)

The requirements for system suitability are usually developed after method development and validation have been completed. The criteria selected will be based on the actual performance of the method as determined during its validation. The

following parameters that can be used to determine system suitability prior to analysis.

1) Retention Time

Chromatographic retention is to measure the time between the injection point and maximum of the detector response for correspondent compound. This parameter called "retention time" (t_R). Retention time, t_R is inversely proportional to the eluent flow rate.

2) Resolution

The degree of separation or resolution of two adjacent bands is defined as the distance between band peaks (or centers) divided by the average band width and bandwidths are measured in units of time.

$$Rs = \frac{tR_2 - tR_1}{0.5\ (w_2 + w_1)}$$

3) Theoretical Plates

A column may be considered as being made up of a large number of theoretical plates where distribution of sample between stationary and mobile phase occurs. The number of Theoretical plates (n) in a column is given by the relationship:

$$n = 5.54(t_R / w_{1/2})^2$$

Where,

t_R = retention time of substance

$W_{1/2}$ = width of the peak at half height, obtained directly by electronic integrators

The value of 'n' depends upon the substance being chromatographed as well as the operating conditions such as mobile phase, temperature etc.

Limit = n > 2000 is desirable.

4) Height Equivalent to a Theoretical plate (HETP)

A theoretical plate can be of any height, which decides the efficiency of separation. If HETP is less, the column is more efficient. If HETP is more, the column is less efficient. The height equivalent to a theoretical plate (HETP) is given by

$$HETP = \frac{\text{Length of column}}{N}$$

Where,

n = No. of theoretical plates

5) Capacity Factor (K')

It is the measure of a sample peak in the chromatogram being specific for a given compound, a parameter which specifies the delay of a substance to be separated.

$$K' = \frac{t_r - t_o}{t_o}$$

Where

t_r = retention time measured from time of injection to time of elution of peak maximum

t_o = void volume

Generally the value of K' is > 2

5) Peak Asymmetry

If k′ is higher at low concentrations a situation often prevailing in adsorption chromatography, the low concentration wings of bands will move more slowly than the high concentration parts, and as an initially symmetric band moves down the column it will become skewed and eventually tailed. The result is a band with a sharp front and a long, more or less exponential tail. Asymmetrical peaks can also result from extra column effects, particularly injection problems. In fact, peak symmetry can be used as a criterion of column performance. A very simple comparison of the back half width, *b*, of the peak at 10% of the peak height, divided by the corresponding front half-width, *a*, gives the asymmetry factor :

$$AF = \frac{b}{a}$$

6) Tailing Factor (T)

The tailing factor T, a measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced. In some cases, values less than unity may be observed. As peak asymmetry increases, integration, and hence precision becomes less reliable.

$$T = \frac{W_{0.05}}{2f}$$

Where,

$W_{0.05}$ = width of peak at 5% height

f = Distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline.

Limit: ≤ 2 is preferable.

1.6 STATISTICAL PARAMETERS

Linear regression (Mendham *et al*., 2002)

Once a linear relationship has been shown to have a high probability by the value of the correlation coefficient 'r', then the best straight line through the data points has to be estimated. This can often be done by visual inspection of the calibration graph, but in many cases it is far more sensible to evaluate the best straight line by linear regression (the method of least squares).

The equation of straight line is

$$y = mx + c$$

Where, y the dependent variable is plotted as result of changing x, the independent variable.

To obtain the regression line 'y on x' the slope 'm' of the line and the intercept 'c' on the y axis are given by the following equation.

$$m = \frac{N \Sigma xy - (\Sigma x)(\Sigma y)}{N \Sigma x^2 - (\Sigma x)^2}$$

and

$$c = \frac{(\Sigma y)(\Sigma x^2) - (\Sigma x)(\Sigma xy)}{N \Sigma x^2 - (\Sigma x)^2}$$

Correlation co-efficient (Gary D Christian, 2005)

The correlation co-efficient is used as a measure of the correlation between two variables. When variables x and y are correlated rather than being functionally related. The Pearson correlation co efficient is one of the most convenient to calculate. This is given by,

$$r = \frac{n \Sigma x_1 y_1 - \Sigma x_1 y_1}{\{[n \Sigma x_1^2 - (\Sigma x_1)^2] [n \Sigma y_1^2 - (\Sigma y_1)^2]\}^{1/2}}$$

Where n is the number of data points.

The maximum value of r is 1. When this occurs, there is exact correlation between the two variables. When r is zero, there is complete independence of the variables. The minimum value of r is -1. A negative correlation co-efficient indicates that the assumed dependence is opposite to what exists and is therefore a positive co-efficient for the revered relation. The fit must be quite poor before r becomes smaller than about 0.98 and is really very poor when less then 0.9.

Standard deviation (Underwood, 2006)

It is commonly used in statistics as a measure of precision and is more meaningful than is the average deviation. It may be thought of as a root-mean-square deviation of values from their average and is expressed mathematically as

$$S = \sqrt{\frac{\sum_{i=1}^{i=n} (x_i - \bar{x})}{N - 1}}$$

Where,

S = Standard deviation

If N is large (50 or more) then of course it is immaterial whether the term in the denomination is N -1 or N

Σ = Sum

$\bar{x}$ = Mean or arithmetic average

$x_i - \bar{x}$ = Deviation of a value from the mean

N = Number of observations

Percentage relative standard deviation (% RSD)

It is also known as coefficient of variation (CV). It is defined as the standard deviation (S.D) expressed as the percentage of mean.

$$\text{CV or \% RSD} = \frac{\text{S.D}}{\bar{x}} \times 100$$

Where,

S.D = Standard deviation

$\bar{x}$ = Mean or arithmetic average

The variance is defined as S^2 and is more important in statistics than S itself. However, the latter is much more commonly used with chemical data.

Standard error of mean (S.E)

Standard error of mean can be defined as the value obtained by division of standard deviation by square root of number of observations. It is mathematically expressed as,

$$\text{S.E.} = \frac{\text{S.D.}}{\sqrt{n}}$$

Where,

S.D = Standard deviation

n = number of observations

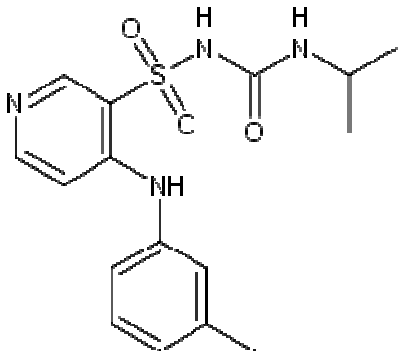
DRUG PROFILE

2. LITERATURE REVIEW

2.1 DRUG PROFILE

TORSEMIDE (THE MERCK INDEX, 2006)

Structure



Molecular Formula

C_{16} H_{20} N_4 O_3 S

Chemical Name

N-[(isopropyl amino) carbonyl]-4-[(3-methyl phenyl) amino] pyridine-3-sulfonamide

Molecular Weight

348.241 g / mol

Protein binding

Highly bound (>99%)

Half life

3.5 hours

Routes

Oral, IV

Category

Torsemide is a pyridine-sulfonylurea type loop Diuretic mainly used in the management of oedema associated with congestive heart failure. It is also used at low doses for the management of hypertension.

Description

White to off white powder

Storage

Stored in tightly closed container

Pharmacological action

Torsemide is a new loop diuretic that potentially may have renal tubular effects from both the blood and urinary sides of the nephron.

Side effects

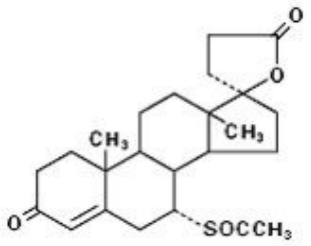
Torsemide can cause orthostatic hypotension, gastro intestinal disturbances, muscle cramps, skin rashes, dizziness, tiredness, and headache. All side effects usually are mild and transient.

Melting point

163-164·c

SPIRONOLACTONE (THE MERCK INDEX, 2006)

Structure



Molecular formula

C_{14} H_{32} O_4 S

Chemical Name

7α-Acetylthio-3-oxo-17α-pregn-4-ene-21, 17-carbolactone

Molecular weight

416.574 g /mol

Half life

10 minutes

Routes

Oral

Category

Loop diuretic

Description

Fine white powder

Storage

Well closed tight container

Mechanism of action

Spironolactone inhibits the effects of aldosterone by Competing for intracellular aldosterone receptors in the distal tubule cells.

Adverse effects

Spironolactone is associated with an increased risk bleeding from the stomach and duodenum, but a causal relationship between the two has not been established. Because it also affects androgen receptor and other steroid receptors.

Identification test

Shake about 10 mg with 2 ml of *sulphuric acid (50%);* an orange solution with an intense yellowish fluorescence is produced. Heat the solution gently; the colour becomes deep red and hydrogen sulphide is evolved which turns litmus paper blue. Add the solution to 10 ml of *water*; a greenish yellow solution is produced which shows opalescence or a precipitate.

Specific optical rotation:

Between -33^{o} and -37^{o}, determined in a 1% w/v solution in *chloroform.*

Loss on drying

Not more than 0.5%, determined on 1 g by drying in an oven at 105^{o} for 3 hours.

2.2 REPORTED METHODS

1. **Dunn C J *et al.,* (1995)**

Torsemide is a pyridine- sulfonylurea type loop diuretic. Compared to other loop diuretics, Torsemide has a more prolonged diuretic effect equipotent to the dose of furosemide and relatively decreased potassium loss. There is no evidence of Torsemide – induced ototoxicity demonstrated in humans.

2. **Melissa young *et al.,* (2001)**

Torsemide is a loop diuretic used for the treatment of hypertension and for oedema in chronic heart failure (CHF), renal failure and cirrhosis.

3. **Senzaki H. *et al.,* (2008)**

To examine the efficacy and safety of Torsemide in children with chronic heart failure. Torsemide can be safely used and appears to be effective for treatment of HF in children. Future clinical trials are warranted to verify the present results.

3. AIM AND PLAN OF WORK

AIM OF WORK

The prime importance of drug analysis is to gain information about the qualitative and quantitative composition of substance and chemical species, that is to find out what a substance is composed of and exactly how much.

This information guides development of the manufacturing operations and therapeutic action of drugs.

Standard analytical procedure for newer drugs or formulation may not be available in pharmacopoeias. Hence it is essential to develop newer analytical methods which are accurate, precise, specific, linear, simple and rapid.

Torsemide and Spironolactone is a newer combination. It has been launched in the market recently.

Hence the present study aims to develop simple, precise and accurate methods for the determination of Torsemide and Spironolactone by simple UV methods and RP-HPLC method in bulk and in formulation.

PLAN OF WORK

- The extensive survey of literature for Torsemide and Spironolactone regarding their physio chemical properties, pharmacological properties and analytical methods. This formed the basis for the development of methods.
- Identification, selection and collection of Torsemide and Spironolactone for analysis.
- Selection of suitable solvent for quantitative extraction of drug present in the formulations. The solvent should be readily available, economical and of analytical grade for UV-spectroscopy and HPLC grade for RP-HPLC and should not chemically interact with the compound of interest and its structural characteristics.
- Selection of suitable method for analysis depending upon the spectral characteristics of the drug.
- Selection of suitable wavelength for rapid and accurate simple UV spectroscopic methods development.
- Development of rapid and accurate RP-HPLC method by using UV detector.
- Analysis of marketed tablet formulation by the proposed methods.
- Statistical analysis of developed analytical methods.
- Validation of analytical methods as per the ICH guidelines.

Materials

And

Methods

4. MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 Drug Sample

Torsemide and Spironolactone as working standard was obtained as a gift sample from Dr. Reddy's Laboratories, Hyderabad.

4.1.2 Formulations Used

TORLACTONE (Sun Pharmaceuticals Ltd., Mumbai) and DYTOR+ (Cipla Ltd) containing the formulation was procured from the local market.

4.1.3 Chemicals and Solvents used

All the following chemicals used were of analytical and HPLC grade.

1. 0.1 N HCl
2. Methanol
3. Distilled water
4. Acetonitrile (HPLC grade)
4. Water for HPLC
5. Potassium Dihydrogen phosphate
6. Ortho phosphoric acid

Chemicals and solvents are procured from Qualigens India Pvt. Limited and Loba Chemie India Limited.

4.1.4 Instruments Used

Instruments employed for the study were,

- SHIMADZU AUX - 220 Digital Balance
- SHIMADZU – 1700 Double Beam - UV – Visible spectrophotometer with pair of 10 mm matched quartz cells.

- SHIMADZU HPLC

  - SPD – IO A_{VP} UV – VIS detector

  - LC – 10 AT*VP* solvent delivery module

- ELICO SL 159 Spectrophotometer
- ELICO pH meter (Model LI - 120)
- Melting point apparatus

4.1.5 Instruments Specifications

A) Shimadzu AUX – 220 digital balance: (Shimadzu instruction manual)

Specifications	
Weighing capacity	200 gms
Minimum display	0.1 mg
Standard deviation	≤ 0.1 mg
Operation temperature range	5 to 40° C

B) Shimadzu UV – Visible spectrophotometer: (Shimadzu instruction manual)

Model: Shimadzu, UV-1700; Cuvetts: 1 Cm quartz cells.

Specifications	
Light source	20 W halogen lamp, Deuterium lamp. Light source position automatic adjustment mechanism.
Monochromator	Aberration-correcting concave holographic grating
Detector	Silicon Photodiode
Stray Light	0.04% or less (220 nm: NaI 10 g/l) 0.04% or less (340 nm: $NaNo_2$ 50 g/l)
Measurement wavelength range	190~1100 nm
Spectral Band Width	1 nm or less (190 to 900 nm)
Wavelength Accuracy	± 0.5 nm automatic wavelength calibration mechanism

Recording range	Absorbance : -3.99~3.99 Abs Transmittance : -399~399%
Photometric Accuracy	± 0.004 Abs (at 1.0 Abs), ±0.002 Abs (at 0.5 Abs)
Operating Temperature/Humidity	Temperature range : 15 to 35°C Humidity range : 35 to 80% (15 to below 30° C) 35 to 70% (30 to 35° C)

C) Shimadzu High Performance Liquid Chromatography: (Shimadzu instruction manual)

Detector Specifications	
Light source	Deuterium Arc lamp
Measurement wavelength range	190 to 700 nm
Spectral Band Width	5 nm
Wavelength Accuracy	± 1 nm
Cell path length	10 nm
Cell volume	20 µl
Operating temperature range	4 to 35° C (39 to 104° F)
Recording range	0.0001 to 4.000 AUFS
Operating temperature/Humidity	4 to 35° C / 75 %
Pump Specifications	
Pump type	Double reciprocating plunger pump
Pumping method	Constant flow delivery and constant pressure delivery
Suction filter	45 µm
Line filter	5 µm mesh
Operating temperature	4 to 35° C (39 to 104° F)

4.2 METHODOLOGY

In the present work an attempt was made to develop and validate a simple, precise and accurate methods for the estimation of Torsemide and Spironolactone in pure and in combined tablet dosage forms by UV- spectrophotometry and RP-HPLC methods.

The drugs were identified by checking the melting point of Torsemide and performing identification test for Spironolactone.

4.2.1. UV-Spectrophotometry

A. Simultaneous equation method

B. Absorbance ratio method

C. Derivative spectrophotometric method

4.2.1.1 UV-Spectrophotometric method for the estimation of Torsemide

4.2.2 RP-HPLC

SPECTROSCOPIC METHODS

4.2.1 SELECTION OF SOLVENT

The solubility and stability for both Torsemide and Spironolactone were evaluated. The absorbance of both drugs were higher and exhibited distinct λmax in methanol followed by 0.02M phosphate buffer (pH-3.5) in the final dilution, since it was decided to prepare drug solutions in methanol followed by 0.02M phosphate buffer (pH-3.5).

PREPARATION OF STANDARD STOCK SOLUTION

Pure raw materials, Torsemide 100 mg and Spironolactone 100 mg were accurately weighed and dissolved separately in methanol to produce 1000 mcg/ml solution.

SELECTION OF WAVELENGTH

The selection of wavelength for the estimation of Torsemide and Spironolactone, a suitable standard solution to contain 10 mcg/ml of Torsemide and Spironolactone were prepared individually and scanned in the entire range from 200-400 nm, an overlain spectra was made. From the spectra, the λmax of Torsemide was found to be 289 nm and λmax of Spironolactone was found to be 242.5 nm. The Isobestic point was found to be 260.5 nm. Hence, the λmax of two drugs was selected for the simultaneous equation method. For the absorbance ratio method, the λmax of Spironolactone and Isobestic point were used. For Derivative Spectroscopic method, the zero order spectrum was derivatised to first order spectrum in that 310 nm was selected for the estimation of Torsemide, which is zero crossing for Spironolactone and 248.5 nm was selected for the estimation of Spironolactone which is zero crossing for Torsemide.

LINEARITY AND CALIBRATION

The linearity was carried out individually for both the drugs in zero order spectrum and first order spectrum at all above said wavelengths. The Beers law limit was found to be in the range of 4-20 mcg/ml. The calibration curves were obtained by plotting absorbance versus concentration of the standard solution.

QUANTIFICATION IN FORMULATION

Twenty tablets were weighed and average weight was found out and it was finely powdered. Powdered drug equivalent to 25 mg of Spironolactone was transferred in to a 25 ml standard flask and the content of the flask was dissolved in methanol by sonication for 15 minutes and then made up to the required volume. The solution was filtered through whatmann filter paper (No.41).The solution was diluted to get a concentration of 20 mcg/ml using Phosphate buffer (pH-3.5). Absorbance of the diluted sample solutions were measured for the methods as described in above said wavelengths.

VALIDATION

PRECISION

Precision of the method was demonstrated by repeatability studies. Repeatability studies were done by consequently analyzing the sample solution for six times. Intra day and inter day precision were established by repeating the determination on the same day and on different days respectively.

RECOVERY STUDIES

In order to ensure the accuracy of the proposed method recovery studies were carried out. To the pre analyzed sample solution, a definite concentration was added and then its recovery was studied. 1ml of pre analyzed formulation was taken in three separate 10 ml volumetric flasks, with these, known concentration of pure drug (Torsemide and Spironolactone) at 60%, 80% and 100% levels were added. The absorbances of resulting solutions were measured at their corresponding wavelength and the percentage recovery was calculated.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

Preparation of calibration curve from the serial dilution of standard was repeated for six times. The limit of detection and limit of quantification was calculated by using the average value of slope and standard deviation.

4.2.1.1 SPECTROSCOPIC METHOD FOR THE ESTIMATION OF TORSEMIDE

To compare different brands of Torsemide a new analytical method has been developed

SELECTION OF SOLVENT

The solubility of Torsemide was determined in a variety of solvent. From the solubility studies 0.1N HCl was selected as a solvent based on stability of Torsemide.

SELECTION OF λMAX

The standard stock solution of Torsemide in 0.1N HCl was prepared. The standard stock solution was further diluted to get 10 mcg/ml solution. The solution was scanned between 200-400 nm wavelength range using 0.1N HCl as blank. From the UV spectra, 286.5 nm was selected as λmax for the analysis of Torsemide.

LINEARITY AND CALIBRATION CURVE

4 ml of stock solution (contains 1000 mcg/ml) was pipetted out into a 100 ml standard flask and the volume was made up to the mark with 0.1N HCl to get a concentration of 40 mcg/ml solution. The aliquots (1-8 ml) of working standard solution of Torsemide were transferred into a 10 ml standard flask and made up to the mark with 0.1N HCl. The absorbance was measured at 286.5 nm against 0.1N HCl as blank. The sample solutions were found to be linear from 4-32 mcg/ml. The calibration curve was plotted between concentration and absorbance.

QUANTIFICATION IN FORMULATIONS

Four formulations were selected and twenty tablets of each formulation [(Torsinex 10mg), (Henletor 20mg), (Tide 10 mg) and (Dytor10 mg) were weighed to find out the average weight and powdered well. Transferred the powdered tablets equivalent to 25 mg of Torsemide into a 25 ml volumetric flask, sonicated for 15 minutes and made up to the mark with 0.1N HCl. Half of the solution was filtered using whatmann filter paper No.41. The absorbance was measured at 286.5 nm using 0.1 N HCl. The amount of Torsemide present in each formulation was calculated by using the slope and intercept values.

RECOVERY STUDIES

From each formulation of the pre-analyzed formulation, known quantities were taken and the raw material solution was added in ascending amount to 10 ml standard flask. The contents were mixed well, finally made up to the mark and filtered. The recovery studies for each formulation was calculated from slope and intercept values.

4.2.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

Chromatographic method depends up on the nature of the sample, molecular weight and solubility. The drug selected for the present study was polar compound; hence it can be separated either by normal phase or reverse phase chromatography. Reverse phase chromatographic technique was selected for initial separations with the knowledge of properties of compound, C_{18} column was chosen as stationary phase and a various ratios of phosphate buffer (pH 3.5), methanol and acetonitrile commonly considered as mobile phase.

SELECTION OF MOBILE PHASE AND λMAX

Different mixtures of mobile phase with different ratios were selected like Acetonitrile: water (60:40), Phosphate buffer: methanol (60:40) and Phosphate buffer: Acetonitrile: methanol (40:40:20) and their chromatograms were recorded in Fig-6, 7

and 8. The above said mobile phase have not given sharp elution, but Phosphate buffer (pH 3.5): Acetonitrile: Methanol (40:30:30) was selected as a mobile phase since these two drugs were eluted with sharp peak and with better resolution. Hence this mobile phase was used to optimize the chromatographic conditions.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

The following parameters were used for RP-HPLC analysis of Torsemide and Spironolactone

Mode of operation	- Isocratic
Stationary phase	- C_{18} column (150mm × 4.6 mm I, d., 5m)
Mobile phase	- Phosphate buffer (pH 3.5): Methanol: Acetonitrile
Ratio	- 40:30:30
Detection wavelength	- 262
Flow rate	- 1 ml/min
Temperature	- Ambient
Sample volume	- 20 mcg/ml
Operating pressure	- 150 kgf

PREPARATION OF THE STANDARD STOCK SOLUTION

Weighed accurately 25 mg of Torsemide and Spironolactone, transferred into a 25 ml standard flask separately and dissolved with minimum quantity of methanol and the volume was made up to the mark with methanol. From the above solutions, 1 ml was transferred into a 10 ml flask to get the concentration of 100 mcg/ml.

LINEARITY AND CALIBRATION

From the standard solution, pipetted 0.5-2.5 ml into a series of five 10 ml flask and made up to the mark with mobile phase to obtain the concentration range from 5-25 mcg/ml and the calibration curve was plotted between concentration and peak area.

QUANTIFICATION OF TORSEMIDE AND SPIRONOLACTONE

Twenty tablets containing 10 mg of Torsemide and 50 mg of Spironolactone were accurately weighed and powdered tablets equivalent to 25 mg of Spironolactone was transferred to a 25 ml volumetric flask and dissolved in methanol and sonicated for 15 minutes. The final concentration was 1000 mcg/ml. From the above solution, a part was filtered through whatmann filter paper and the clear solution was collected, 2.5 ml was pipetted into a 10 ml volumetric flask further diluted 1 ml to 10 ml and made up to the mark with mobile phase to produce 25 mcg/ml solution. The peak area measurements were done by injecting sample (20 mcg/ml) six times and the amount of Torsemide and Spironolactone were calculated from the respective calibration curve.

RECOVERY STUDIES

To ensure the reliability of the method, recovery studies were carried out by mixing a known quantity of standard drug solution with the pre-analyzed sample formulation and the content were mixed and made to the volume with mobile phase and re- analyzed by the proposed method, the percentage recovery was calculated.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

Preparation of calibration curve for the serial dilution of standard was repeated for six times. The limit of detection and limit of quantification were calculated by using the average value of slope and standard deviation.

SYSTEM SUITABILITY STUDIES

The system suitability studies were carried out as specified in U.S.P. the parameter like column efficiency, tailing factor, asymmetric factor, theoretical plate number and capacity factor were calculated.

RESULTS

AND

DISCUSSION

5. RESULTS AND DISCUSSION

ABOUT UV SPECTROSCOPIC METHODS

In order to quench the thirst for the analysis of a newer drug, the drug Torsemide and Spironolactone were taken for our studies. To ensure the percentage purity of Torsemide and Spironolactone in combined dosage form and various brands of Torsemide, the UV- spectroscopic methods were developed. The methods were very simple, economic and applicable for routine analysis.

5.1 UV-SPECTROSCOPIC STUDIES

The solubility of Torsemide and Spironolactone were determined in a variety of solvents using Schefter and Higuchi method. 10 mg of sample was taken in test tubes and checked with variety of solvents and their solubility profiles have been shown in Table-1.

From the solubility studies, methanol followed by phosphate buffer (pH-3.5) was chosen as a solvent, for UV- spectroscopic studies in bulk and in formulations. Based upon its easy availability, cost factor and the stability conditions, the solvents were selected for the method.

Three simple, sensitive and precise UV methods namely, Simultaneous Equation method, Absorbance Ratio method and Derivative Spectroscopic methods were preferred for the determination of Torsemide and Spironolactone in Pharmaceutical formulations.

The drug was dissolved in methanol and followed by buffer to produce 10 mcg/ml. It was scanned in the range of 200-400 nm and it shows constant λmax at 289 nm for Torsemide and 242.5 nm for Spironolactone and overlain spectra was made. This is shown in Fig-1, 2 and 3. Stability of absorbance at their λmax was also checked.

The linearity of Torsemide and Spironolactone was constructed in the range of 4-20 mcg/ml and its calibration curve is shown in the Fig-4 and 5. The optical characteristics such as Beer's laws limit (4-20 mcg/ml), molar extinction co-efficient, Sandell's sensitivity, correlation co-efficient, slope and intercept were calculated and are shown in Table-2.

The Limit of detection and the Limit of quantification were determined from the linearity studies which was done 6 times and calculated by using slope and standard deviation.

The formulations, Torlactone and Dytor+ were selected for analysis. The amount present were determined by average of six replicate analysis and the percentage purity was found to be in the range of 98.2-101.4% by all the three methods and it is shown in Table- 3, 4 and 5.

To evaluate the accuracy of the method, recovery studies were carried out, known amount of pure drug was added to the previously analyzed solution containing formulation and the mixture was reanalyzed by the proposed method and the recoveries were calculated. The percentage recovery of Torsemide and Spironolactone in the formulations viz Torlactone and Dytor+ were found to be in the range of 99.8-102.4%. Values are given in Table-6, 7 and 8.

Precision of the method was studied by making repeated analysis of the same sample and it was carried out three times in a day for three days. The % RSD and standard deviation for inter-day and intra day analysis was found to be less than 2 indicates the method is precise, which are given in Table 9 and 10.

5.1.1 SPECTROSCOPIC METHODS FOR THE ESTIMATION OF TORSEMIDE

The solubility of Torsemide was determined in number of solvents. From the solubility data, 0.1 N HCl was selected as solvent because of its good stability and easy availability.

Different aliquots of Torsemide in 0.1 N HCl was prepared in the concentration range of 4-32 mcg/ml. The absorbance of solution was measured at 286.5 nm.

The calibration curve was plotted using concentration against absorbance. The optical parameters like correlation co-efficient, slope, intercept, Sandell's sensitivity, LOD and LOQ were calculated. These are shown in Table-11.

The correlation co-efficient value for the calibration curve was found to be 0.9999. It indicates that the concentration of Torsemide with 0.1 N HCl has acceptable limit to proceed further studies.

Torsemide containing tablets from four formulations [Torsinex 10 mg, Henletor 20 mg, Tide 10 mg and Dytor 10 mg] were selected for analysis. The nominal concentration of Torsemide was prepared and the absorbances of the solution were measured at 286.5 nm.

The amount of test solution was calculated by using slope and intercept values. This procedure was repeated for six times to ensure the precision of the method.

The percentage label claims of Torsemide present in tablet formulations were found. The % RSD value was found to be ± 0.08412. The low % RSD value indicates that the method has good precision. The results of analysis are shown in the Table-12 to 15.

The accuracy of the method was performed by recovery studies. To the pre-analyzed formulation, a known quantity of Torsemide raw material solution was added.

The absorbance of the solution was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 100.2% to102.3%, with low % RSD values.

It indicates that there is no interference due to excipients used in formulation. Hence the method was found to be accurate. The recovery data were shown in the Table-16 to 19.

5.2 RP-HPLC METHOD

An effort has been made to identify a simple, cost effective, economic, specific and accurate method for the estimation of Torsemide and Spironolactone in formulation.

The mobile phase with different ratios were selected like Acetonitrile: water (60:40), in this mobile phase the Rt is less than 2 minutes. Phosphate buffer: Methanol (60:40), here the peaks are very broad and Phosphate buffer: Acetonitrile: Methanol (40:40:20), these mobile phases have not given sharp elution of both drugs and also they are not in desired system suitability parameters. Hence the ratio was changed to 40:30:30 of Phosphate buffer, methanol, acetonitrile respectively, this gives sharp elution and desired limits.

The mobile phase which consists of Phosphate buffer (pH 3.5): Methanol: Acetonitrile was chosen in the ratio 40:30:30 based on its sharp elution and acceptable system suitability parameters. Detection of Torsemide and Spironolactone in mobile phase was found using UV-Visible detector at 262 nm, the flow rate was kept at 1 ml/min. The retention time with optimum parameters were selected for the analytical studies.

Optimization was done with the above mobile phase and various concentrations of raw material such as 5-25 mcg/ml of Torsemide and Spironolactone were prepared, injected and the chromatograms were recorded. This is shown in the Fig. 9-13. The optical characteristic such as Beer's law limit (5-25 mcg/ml), correlation co-efficient, slope and intercept were calculated and shown in Table-20. The results were found to be satisfactory. The calibration curve for Torsemide and Spironolactone was plotted and shown in Fig-14 and 15.

The limit of detection and the limit of quantification were determined by using slope and Standard Deviation. The LOD and LOQ were calculated.

The system suitability test parameters such as Tailing factor, Theoretical plate per unit length and Asymmetric factor and resolution were calculated for the

concentration of 10 mcg/ml and are shown in Table-21, the parameters were found to be satisfactory as per guidelines.

The formulation Dytor+ was selected for the analysis. The Tablet powder equivalent to 25 mg of Spironolactone was taken and 25 mcg/ml concentration was prepared and injected. Injection repeatability was performed for the same concentration six times and amount present was found to be 100.2 % and 98.5 % for Torsemide and Spironolactone respectively. The values are shown in the Table -22, hence the precision was confirmed by repeatable injection of formulation and their chromatograms are shown in the Fig-16 to 21.

Accuracy was confirmed by recovery studies, by adding known amount of pure drug to the previously analyzed formulation and the mixture was reanalyzed by the proposed method and it is shown in the Fig-22 to 24. The percentage recovery of Dytor+ was found to be 102.2% and 100.5% for Torsemide and Spironolactone respectively. The values are given in Table-23. The proposed method was validated and found that the excipients and additives did not interfering the developed method.

All the above parameters combined with simplicity and easy of operation ensures that the application of the proposed method for the assay of drug in Pharmaceutical dosage forms.

SUMMARY

AND

CONCLUSION

6. SUMMARY AND CONCLUSION

Torsemide is a pyridine-sulfonylurea type loop Diuretic mainly used in the management of oedema associated with Congestive Heart Failure. It is also used at low doses for the management of hypertension. Spironolactone is a powerful aldosterone antagonist.

The proposed analytical methods are simple, economical, rapid, sensitive, reproducible and accurate for the estimation of Torsemide and Spironolactone. The methods adopted for studies were

1. UV- spectroscopic method for the estimation of Torsemide and Spironolactone in combined dosage form by
   - ❖ Simultaneous equation method
   - ❖ Absorbance ratio method
   - ❖ Derivative spectroscopic method
2. UV-spectroscopic method for the estimation of Torsemide in different formulations.
3. RP-HPLC method for the estimation of Torsemide and Spironolactone in combined dosage form.

The drug samples containing Torsemide and Spironolactone in combined dosage form were analyzed by UV-spectroscopic method using methanol followed by 0.02 M Phosphate buffer (pH-3.5) as a solvent and the content of drug present in each formulations (Torlactone and Dytor+) were found to be satisfactory.

The estimation of Torsemide containing tablets by UV-spectroscopic method using 0.1N HCl was done and four formulations were selected [Torsinex 10 mg, Henletor 20 mg, Tide 10 mg and Dytor 10 mg]. The results were found to be satisfactory.

Simultaneously, a newer RP-HPLC method has been developed for the estimation of both drugs in bulk and in formulation. The proposed method gives reliable assay results with short analysis time using mobile phase phosphate buffer

(pH 3.5): Methanol: Acetonitrile in the ratio of 40:30:30 respectively. The content of drug present in the formulation (DYTOR+) was found to be satisfactory and system suitability parameters are in desired limit.

All the above methods do not suffer from any interference due to common excipients. It indicates that the methods were accurate. Therefore the proposed methods could be successfully applied to estimate commercial pharmaceutical products containing Torsemide and Spironolactone.

Thus the above studies and findings are helpful to the analytical chemists to apply the analytical methods for the routine analysis of the analyte in Pharmaceutical dosage forms with the approval of FDA.

FIGURES

FIGURE-1

UV-SPECTRUM OF TORSEMIDE

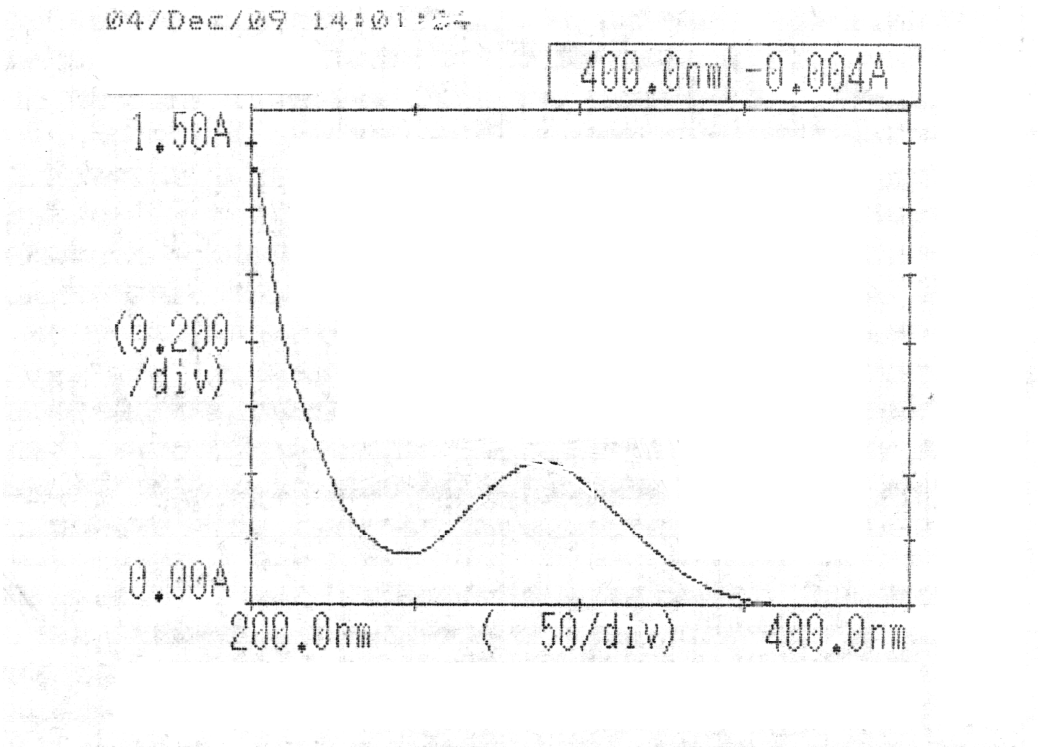


FIGURE-2

UV-SPECTRUM OF SPIRONOLACTONE

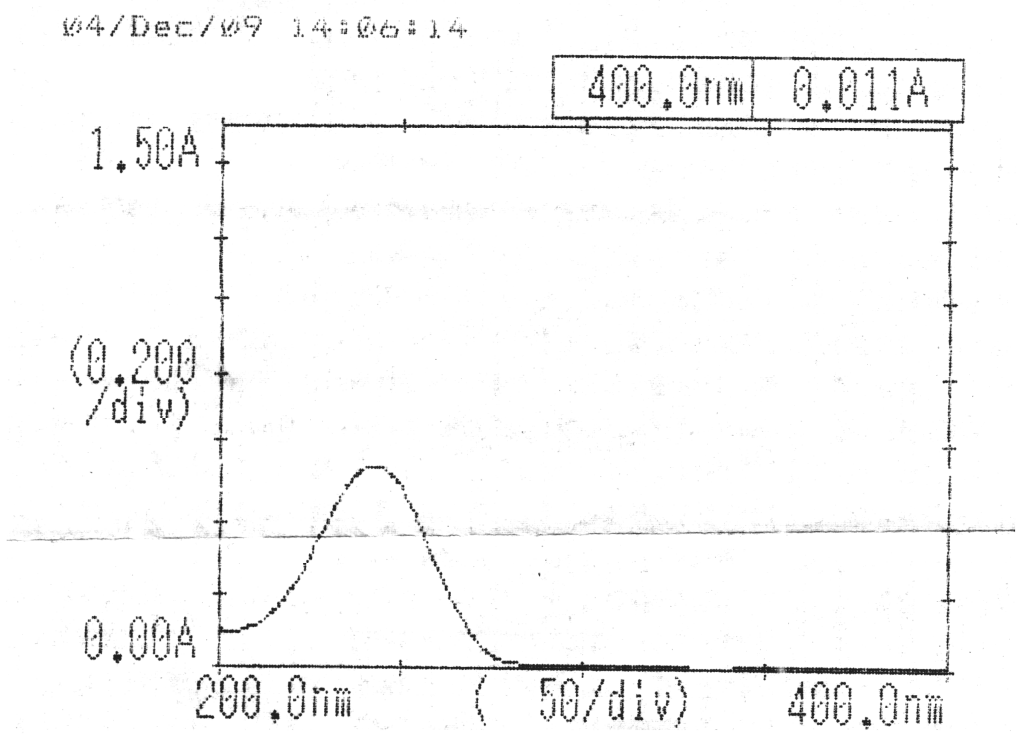


FIGURE -3

OVERLAIN SPECTRUM OF TORSEMIDE AND SPIRONOLACTONE

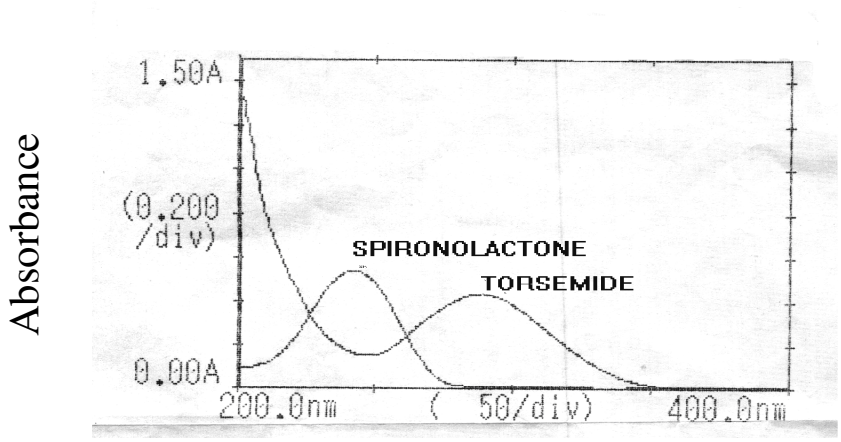


FIGURE-4

CALIBRATION CURVE FOR TORSEMIDE

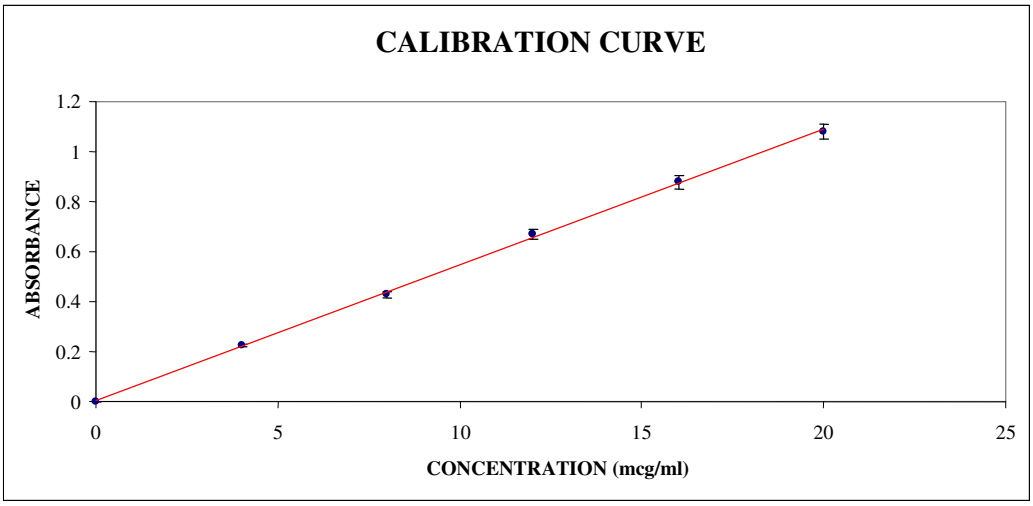


FIGURE-5

CALIBRATION CURVE FOR SPIRONOLACTONE

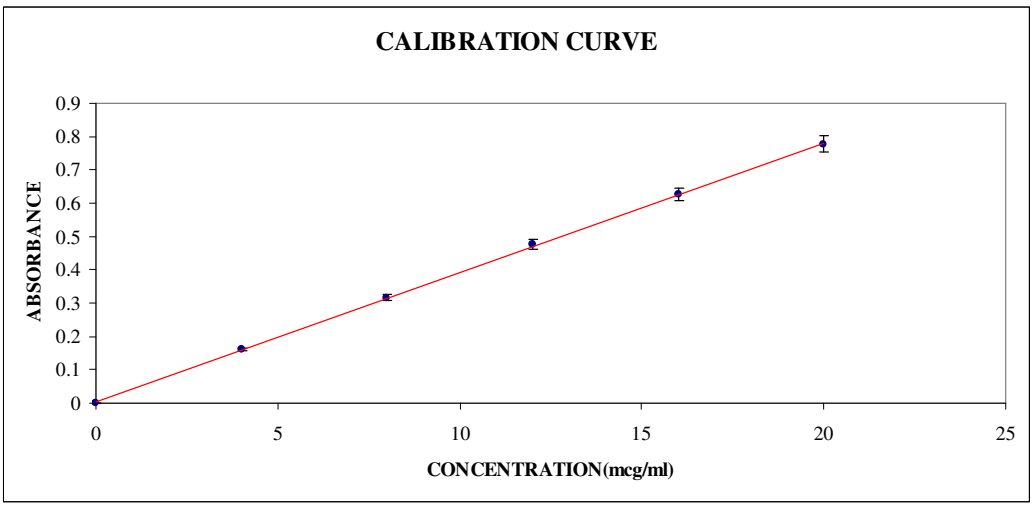


FIGURE-6

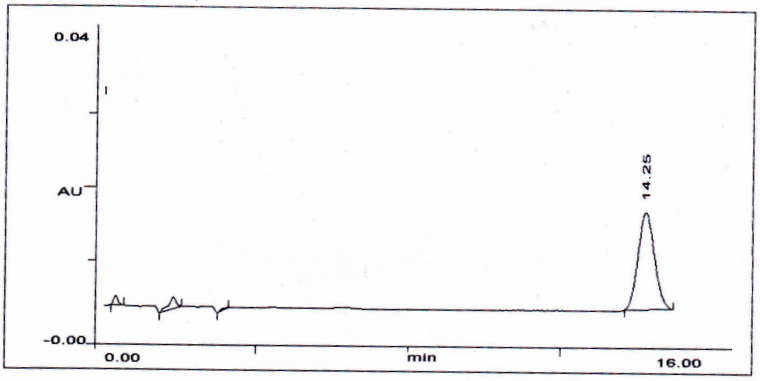
OPTIMIZATION OF CHROMATOGRAM USING PHOSPHATE BUFFER: METHANOL (60:40)

Page No. :1

REPORT

Sample Name: Data File: ...2010\AMG125.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 23 Nov 2009 Time: 14:48:24
Run: ch1: 11
Type of Analysis : Percent On Area and Height
Report printed on : 6/1/2010 at : 09:31:29

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	16.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	14.25	2913	2142317	100.0000	100.0000	BB	0.502
		3e+03	2142317				

Summary :

TOTAL PEAKS : 1

FIGURE-7

OPTIMIZATION OF CHROMATOGRAM USING ACETONITRILE (pH-3): WATER (60:40)

Page No. :1

REPORT

Sample Name: Data File: ...2010\AMG 78.DAT

Method File: AMG.MET

Detector: UV-VIS. System: HPLC

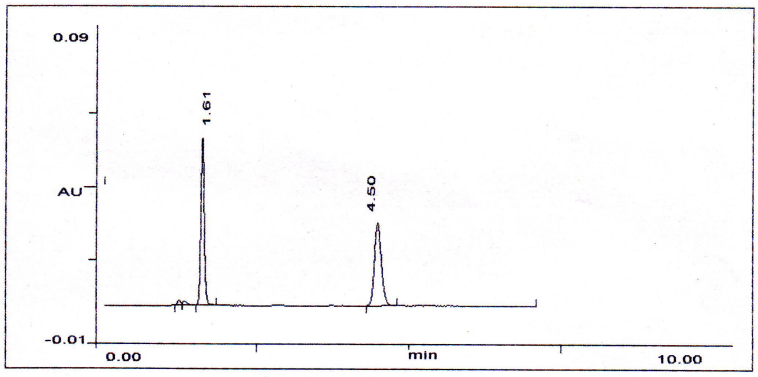
Date: 19 Nov 2009 Time: 14:41:20

Run: ch1: 3

Type of Analysis : Percent On Area and Height

Report printed on : 6/1/2010 at : 09:22:59

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.61	13251	1182159	68.0027	49.9054	VB	0.061
2	4.50	6235	1186641	31.9973	50.0946	BB	0.130
		2e+04	2368800				

Summary :

FIGURE-8

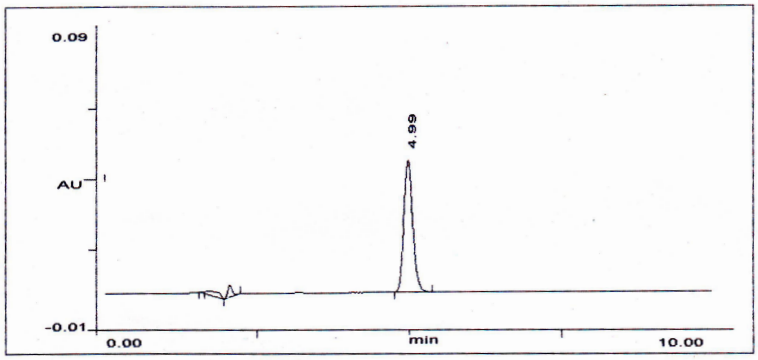
OPTIMIZATION OF CHROMATOGRAM USING ACETONITRILE: METHANOL: PHOSPHATE BUFFER (pH-3.5)

Page No. :1

REPORT

Sample Name: Data File: ...2010\AMG132.DAT
Method File: AMG.MET
Detector: UV-VIS. System: HPLC
Date: 23 Nov 2009 Time: 16:22:20
Run: ch1: 18
Type of Analysis : Percent On Area and Height
Report printed on : 6/1/2010 at : 09:28:14

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	4.99	10358	2557276	100.0000	100.0000	BB	0.169
		1e+04	2557276				

Summary :

TOTAL PEAKS : 1

FIGURE-9

LINEARITY CHROMATOGRAM OF TORSEMIDE AND SPIRONOLACTONE (5 MCG/ML)

REPORT

Sample Name: **Data File: ...om99\VTS 14.DAT**

Method File: MF 1.MET

Detector: UV-VIS. **System: HPLC**

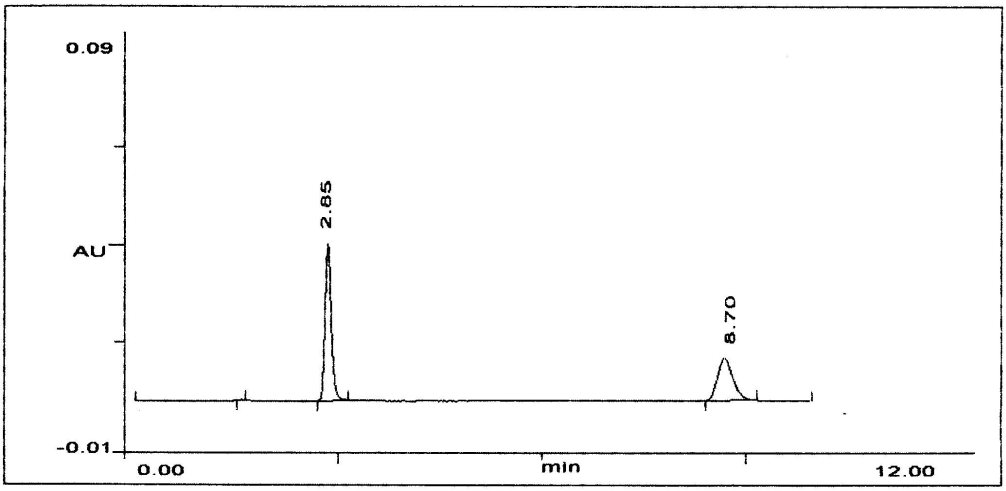
Date: 31 Dec 2009 **Time: 12:18:48**

Run: ch1: 13

Type of Analysis : Percent On Area and Height

Report printed on : 5/1/2010 at : 11:54:13

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.85	9011	1385811	78.7675	59.8074	BB	0.105
2	8.70	2429	931313	21.2325	40.1926	BB	0.262
		1e+04	2317124				

FIGURE-10

LINEARITY CHROMATOGRAM OF TORSEMIDE AND SPIRONOLACTONE (10 MCG/ML)

Sample Name: **Data File: ...om99\VTS 15.DAT**

Method File: MF 1.MET

Detector: UV-VIS. **System: HPLC**

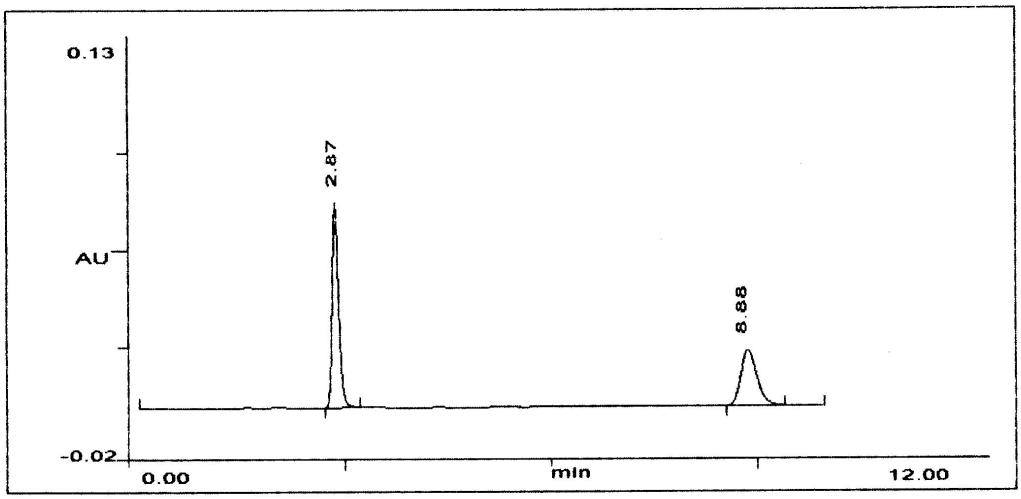
Date: 31 Dec 2009 **Time: 12:29:10**

Run: ch1: 14

Type of Analysis : Percent On Area and Height

Report printed on : 5/1/2010 at : 11:57:20

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.87	17508	2746937	78.6594	59.6728	BB	0.107
2	8.88	4750	1856392	21.3406	40.3272	BB	0.267
		2e+04	4603329				

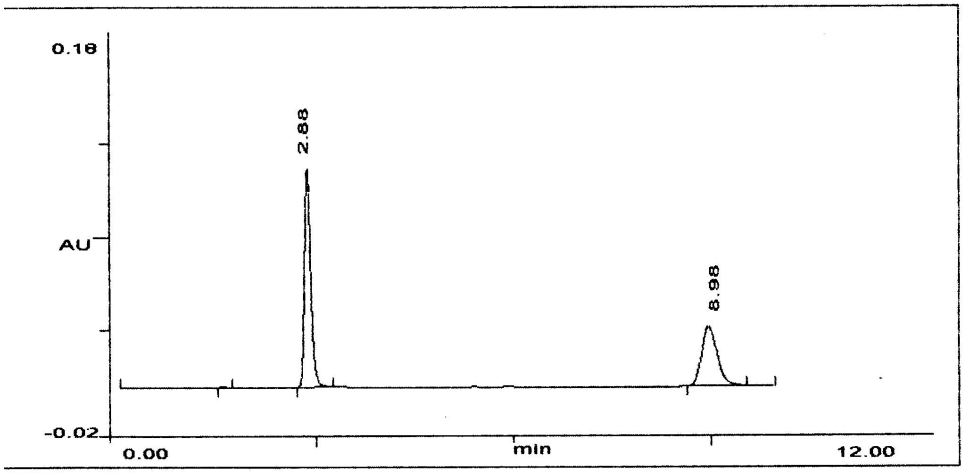
FIGURE -11

LINEARITY CHROMATOGRAM OF TORSEMIDE AND SPIRONOLACTONE (15 MCG/ML)

REPORT

Sample Name: Data File: ...om99\VTS 16.DAT
Method File: MF 1.MET
Detector: UV-VIS. System: HPLC
Date: 31 Dec 2009 Time: 12:39:22
Run: ch1: 15
Type of Analysis : Percent On Area and Height
Report printed on : 5/1/2010 at : 12:01:41

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.88	26510	4194295	78.6904	59.4987	BB	0.108
2	8.98	7179	2855096	21.3096	40.5013	BB	0.271
		3e+04	7049391				

FIGURE-12

LINEARITY CHROMATOGRAM OF TORSEMIDE AND SPIRONOLACTONE (20 MCG/ML)

REPORT

Sample Name: **Data File: ...om99\VTS 17.DAT**

Method File: MF 1.MET

Detector: UV-VIS. **System: HPLC**

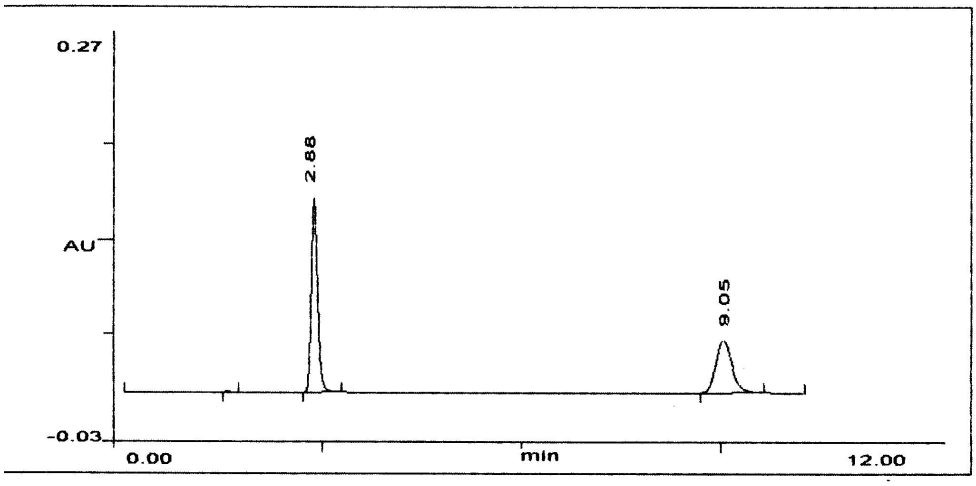
Date: 31 Dec 2009 **Time: 12:49:50**

Run: ch1: 16

Type of Analysis : Percent On Area and Height

Report printed on : 5/1/2010 at : 12:24:21

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.88	34188	5443333	78.7252	59.4178	BB	0.109
2	9.05	9239	3717785	21.2748	40.5822	BB	0.275
		4e+04	9161118				

FIGURE-13

LINEARITY CHROMATOGRAM OF TORSEMIDE AND SPIRONOLACTONE (25 MCG/ML)

REPORT

Sample Name: **Data File: ...om99\VTS 18.DAT**

Method File: MF 1.MET

Detector: UV-VIS. **System: HPLC**

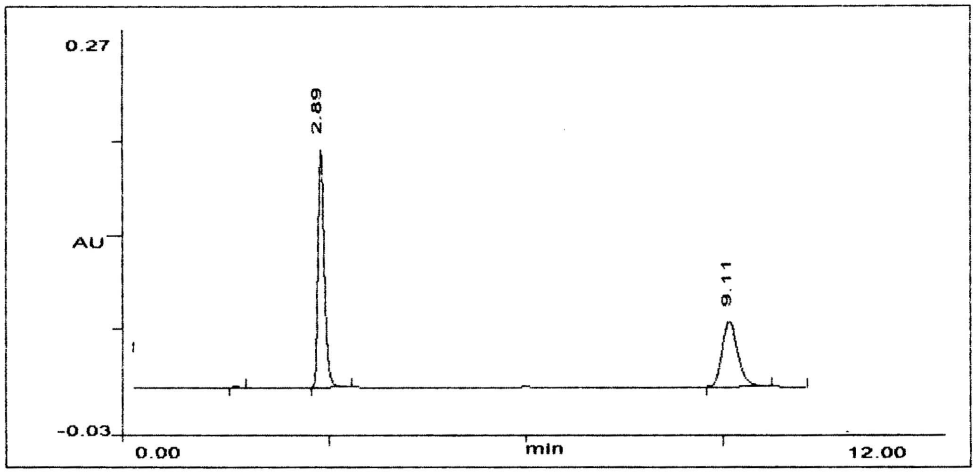
Date: 31 Dec 2009 **Time: 13:0:18**

Run: ch1: 17

Type of Analysis : Percent On Area and Height

Report printed on : 5/1/2010 at : 14:41:07

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.89	42316	6807308	78.5958	59.3787	BB	0.110
2	9.11	11524	4656926	21.4042	40.6213	BB	0.276
		5e+04	11464234				

FIGURE-14

CALIBRATION CURVE FOR TORSEMIDE BY RP-HPLC

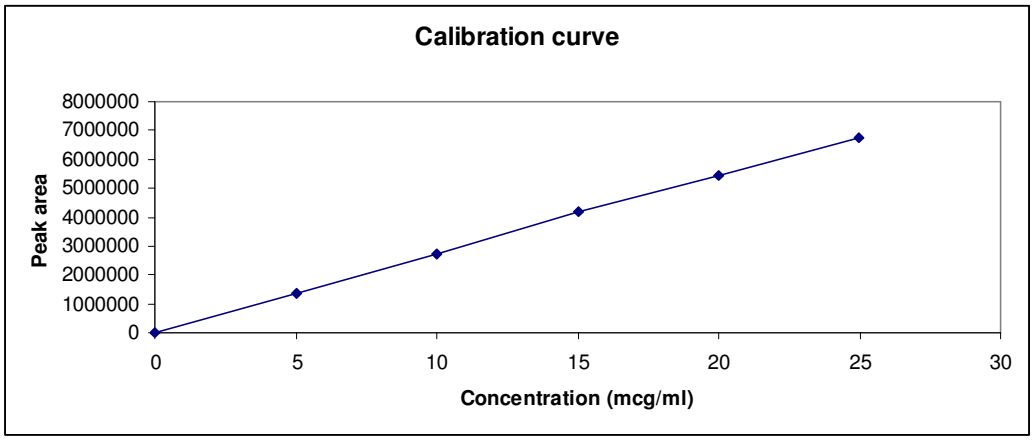


FIGURE-15

CALIBRATION CURVE FOR SPIRONOLACTONE BY RP-HPLC

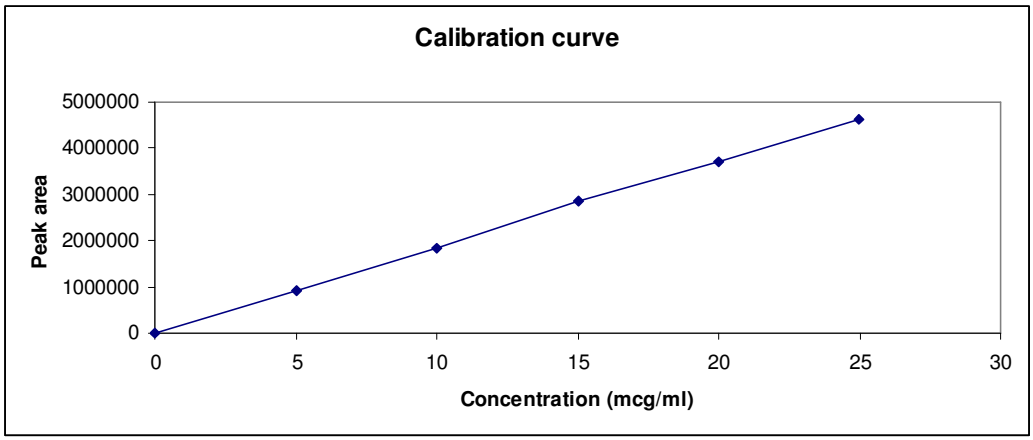


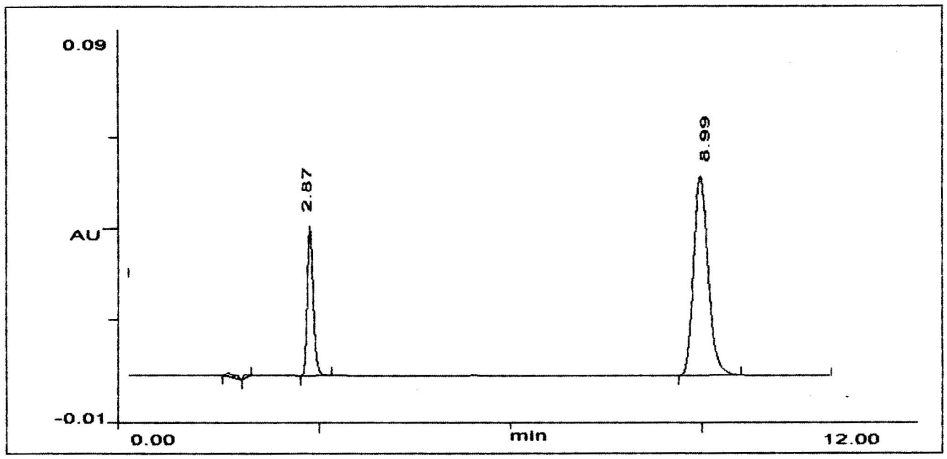
FIGURE-16

QUANTIFICATION OF TORSEMIDE AND SPIRONOLACTONE IN FORMULATION

REPORT

Sample Name:	**Data File: ...om99\VTS 21.DAT**
Method File: MF 1.MET	
Detector: UV-VIS.	**System: HPLC**
Date: 31 Dec 2009	**Time: 13:57:14**
Run: ch1: 20	
Type of Analysis : Percent On Area and Height	
Report printed on : 5/1/2010 at : 16:48:56	

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.87	9132	1452260	42.9620	23.5614	BB	0.109
2	8.99	12124	4711473	57.0380	76.4386	BB	0.265
		2e+04	6163733				

FIGURE-17

QUANTIFICATION OF TORSEMIDE AND SPIRONOLACTONE IN FORMULATION

REPORT

Sample Name: **Data File: ...om99\VTS 22.DAT**

Method File: MF 1.MET

Detector: UV-VIS. **System: HPLC**

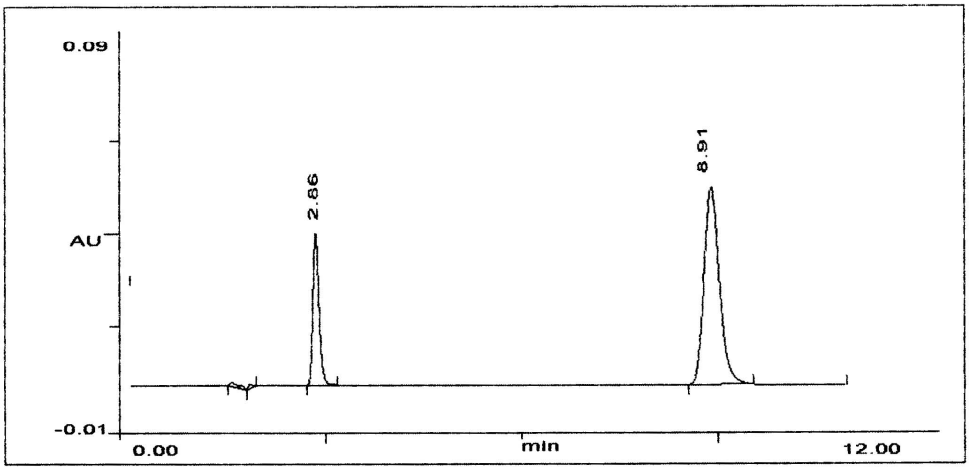
Date: 31 Dec 2009 **Time: 14:8:50**

Run: ch1: 21

Type of Analysis : Percent On Area and Height

Report printed on : 5/1/2010 at : 16:52:28

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.86	9126	1427510	43.8877	23.5448	BB	0.107
2	8.91	11668	4635453	56.1123	76.4552	BB	0.271
		2e+04	6062963				

FIGURE-18

QUANTIFICATION OF TORSEMIDE AND SPIRONOLACTONE IN FORMULATION

REPORT

Sample Name: **Data File: ...om99\VTS 23.DAT**

Method File: MF 1.MET

Detector: UV-VIS. **System: HPLC**

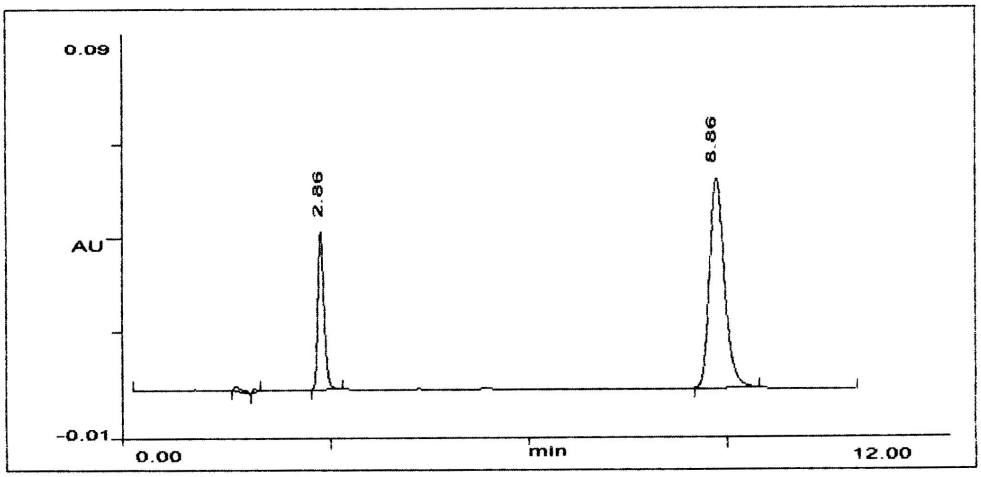
Date: 31 Dec 2009 **Time: 14:20:12**

Run: ch1: 22

Type of Analysis : Percent On Area and Height

Report printed on : 5/1/2010 at : 16:54:13

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.86	9413	1457655	43.1829	23.5408	BB	0.106
2	8.86	12385	4734370	56.8171	76.4592	BB	0.261
		2e+04	6192025				

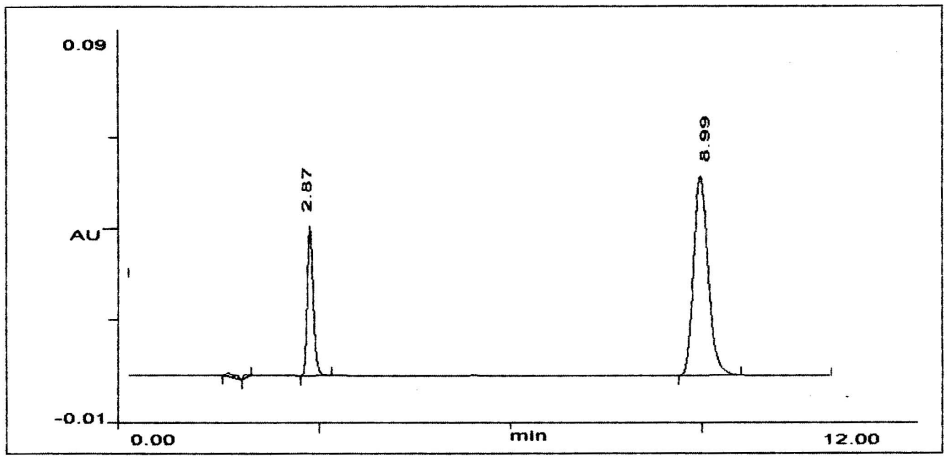
FIGURE-19

QUANTIFICATION OF TORSEMIDE AND SPIRONOLACTONE IN FORMULATION

REPORT

Sample Name:	**Data File: ...om99\VTS 21.DAT**
Method File: MF 1.MET	
Detector: UV-VIS.	**System: HPLC**
Date: 31 Dec 2009	**Time: 13:57:14**
Run: ch1: 20	
Type of Analysis : Percent On Area and Height	
Report printed on : 5/1/2010 at : 16:48:56	

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.87	9132	1452260	42.9620	23.5614	BB	0.109
2	8.99	12124	4711473	57.0380	76.4386	BB	0.265
		2e+04	6163733				

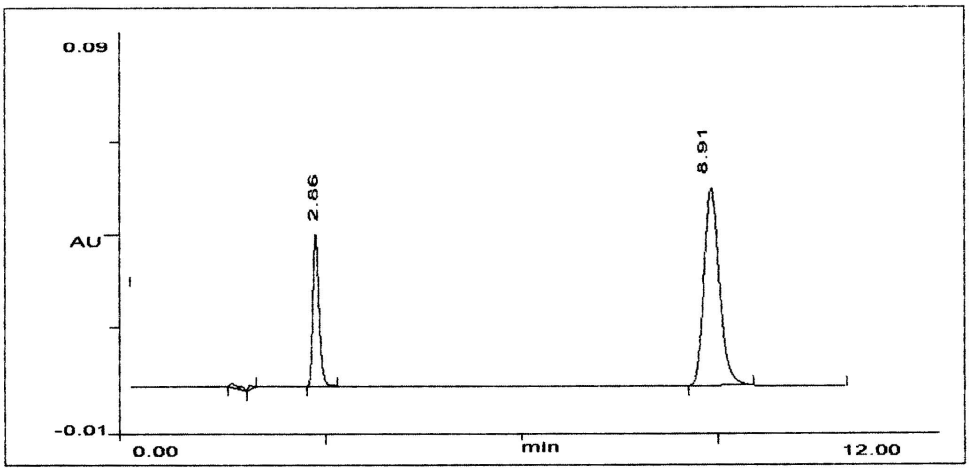
FIGURE-20

QUANTIFICATION OF TORSEMIDE AND SPIRONOLACTONE IN FORMULATION

REPORT

Sample Name:	**Data File: ...om99\VTS 22.DAT**
Method File: MF 1.MET	
Detector: UV-VIS.	**System: HPLC**
Date: 31 Dec 2009	**Time: 14:8:50**
Run: ch1: 21	
Type of Analysis : Percent On Area and Height	
Report printed on : 5/1/2010 at : 16:52:28	

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.86	9126	1427510	43.8877	23.5448	BB	0.107
2	8.91	11668	4635453	56.1123	76.4552	BB	0.271
		2e+04	6062963				

FIGURE-21

QUANTIFICATION OF TORSEMIDE AND SPIRONOLACTONE IN FORMULATION

REPORT

Sample Name: **Data File: ...om99\VTS 23.DAT**

Method File: MF 1.MET

Detector: UV-VIS. **System: HPLC**

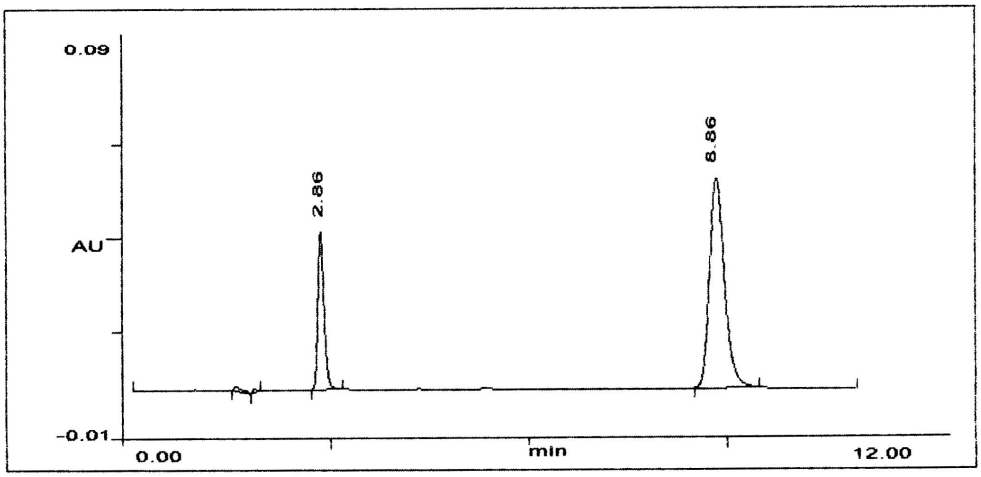
Date: 31 Dec 2009 **Time: 14:20:12**

Run: ch1: 22

Type of Analysis : Percent On Area and Height

Report printed on : 5/1/2010 at : 16:54:13

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.86	9413	1457655	43.1829	23.5408	BB	0.106
2	8.86	12385	4734370	56.8171	76.4592	BB	0.261
		2e+04	6192025				

FIGURE-22

RECOVERY STUDIES OF TORSEMIDE AND SPIRONOLACTONE BY RP-HPLC

REPORT

Sample Name: **Data File: ...om99\VTS 24.DAT**

Method File: MF 1.MET

Detector: UV-VIS. **System: HPLC**

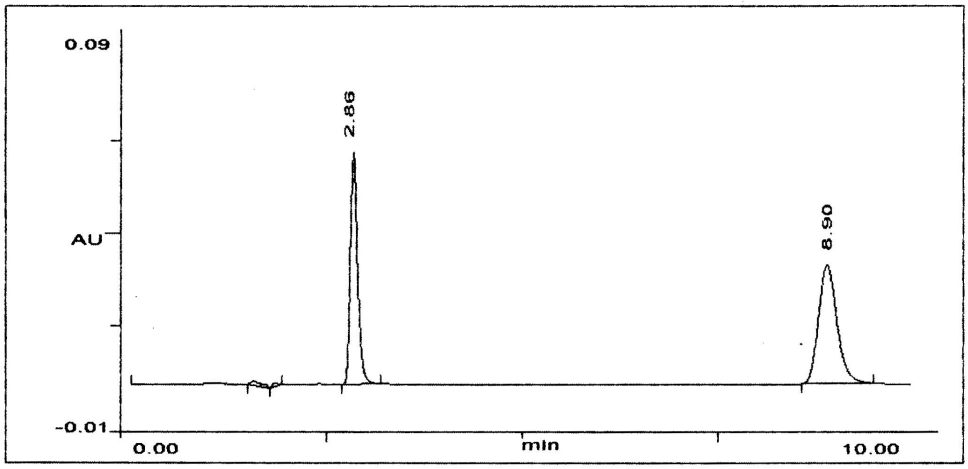
Date: 31 Dec 2009 **Time: 14:31:30**

Run: ch1: 23

Type of Analysis : Percent On Area and Height

Report printed on : 5/1/2010 at : 16:57:14

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.86	13825	2174637	66.3292	43.7316	BB	0.107
2	8.90	7018	2798055	33.6708	56.2684	BB	0.272
		2e+04	4972692				

FIGURE-23

RECOVERY STUDIES OF TORSEMIDE AND SPIRONOLACTONE BY RP-HPLC

REPORT

Sample Name: **Data File: ...om99\VTS 25.DAT**

Method File: MF 1.MET

Detector: UV-VIS. **System: HPLC**

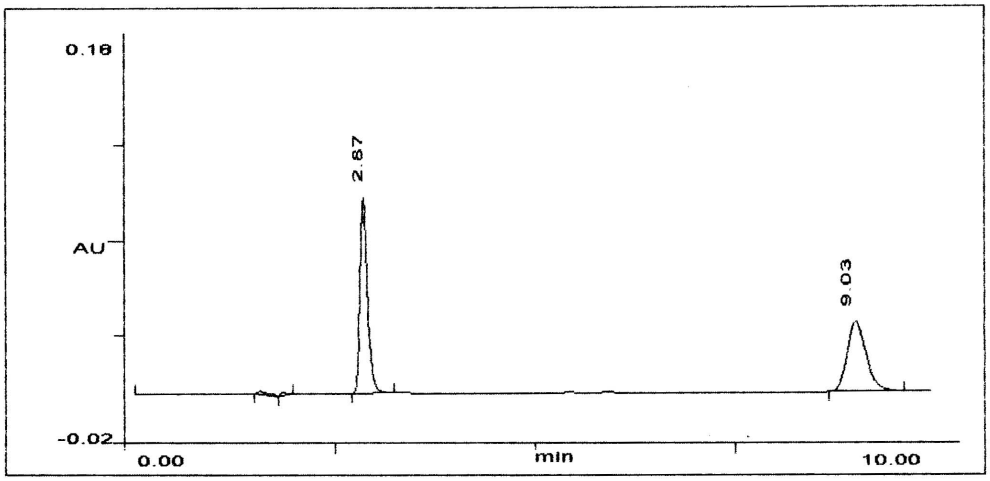
Date: 31 Dec 2009 **Time: 14:42:50**

Run: ch1: 24

Type of Analysis : Percent On Area and Height

Report printed on : 5/1/2010 at : 17:01:58

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.87	22989	3662378	73.6614	52.5082	BB	0.109
2	9.03	8220	3312489	26.3386	47.4918	BB	0.275
		3e+04	6974867				

FIGURE-24

RECOVERY STUDIES OF TORSEMIDE AND SPIRONOLACTONE BY RP-HPLC

REPORT

Sample Name: **Data File: ...om99\VTS 26.DAT**

Method File: MF 1.MET

Detector: UV-VIS. **System: HPLC**

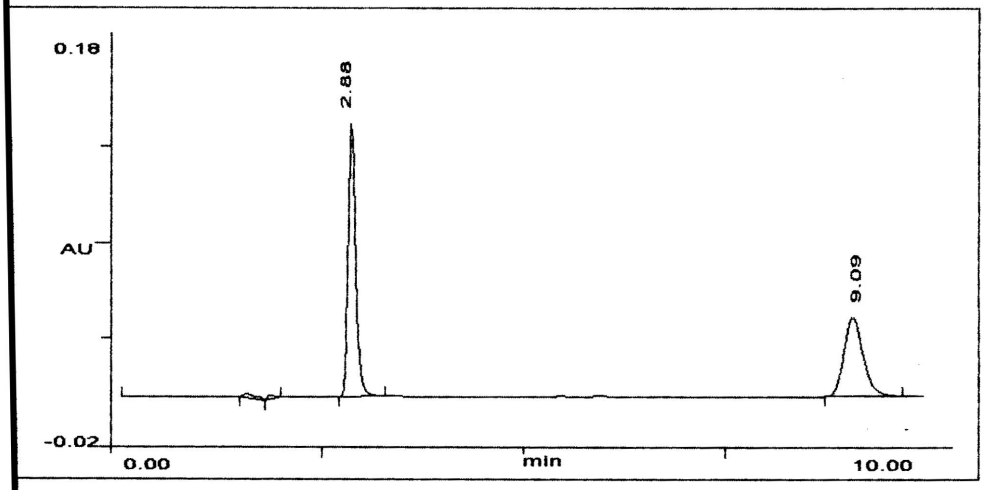
Date: 31 Dec 2009 **Time: 14:54:14**

Run: ch1: 25

Type of Analysis : Percent On Area and Height

Report printed on : 5/1/2010 at : 17:06:39

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0



o.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.88	31649	5078691	77.3833	57.5115	BB	0.110
2	9.09	9250	3752047	22.6167	42.4885	BB	0.277
		4e+04	8830738				

TABLES

TABLE-1

SOLUBILITY OF TORSEMIDE AND SPIRONOLACTONE IN VARIOUS SOLVENTS

SOLVENT	TOR	EXTENT OF SOLUBILITY	SPI	EXTENT OF SOLUBILITY
ACETONITRILE	SOLUBLE	10 mg in 7 ml	SOLUBLE	10 mg in 1 ml
ACETONE	INSOLUBLE	-----	SOLUBLE	10 mg in 1 ml
ACETIC ACID	SOLUBLE	10 mg in 8 ml	SOLUBLE	10 mg in 2 ml
BENZENE	INSOLUBLE	-----	SOLUBLE	10 mg in 3 ml
CHLOROFORM	INSOLUBLE	-----	SOLUBLE	10 mg in 1 ml
ETHANOL	SOLUBLE	10 mg in 5 ml	SOLUBLE	10 mg in 5 ml
0.1 N HCl	SOLUBLE	10 mg in 2 ml	INSOLUBLE	------
0.1 M NAOH	SOLUBLE	10 mg in 6 ml	INSOLUBLE	------
METHANOL	SOLUBLE	10 mg in 4 ml	SOLUBLE	10 mg in 7 ml
WATER	INSOLUBLE	------	INSOLUBLE	--------

TOR is Torsemide
SPI is Spironolactone

TABLE-2

OPTICAL CHARACTERISTICS OF TORSEMIDE AND SPIRONOLACTONE

PARAMETERS	TORSEMIDE	SPIRONOLACTONE
Beer's law limits	4-20 µg/ml	4-20 µg/ml
Molar absorptivity (L/mol/cm)	15248.312	22547.53
Slope (m)	0.043430714	0.052402381
Intercept (c)	0.004697373	0.008902093
Correlation-Co-efficient (r)	0.9998	0.9999
Sandell's sensitivity ($mg/cm^2/0.001A.v$)	0.023025183	0.019091411
LOD	0.356920928	0.560602522
LOQ	1.081578569	1.698795523
Standard Error	0.001676174	0.0004120123

TABLE-3

ASSAY OF COMMERCIAL FORMULATION BY UV- SPECTROSCOPY (SIMULTANEOUS EQUATION METHOD)

Formulation	Drug	S.No.	Amount Labeled (mg/tab)	Amount Estimated (mg/tab)	% Amount found	S.D. (+/-)	R.S.D.	S.E.
TORLACTONE	TOR	1	10	9.836	98.36	0.977	0.978	0.398
		2		9.993	99.36			
		3		9.774	97.74			
		4		10.59	105.9			
		5		10.11	101.1			
		6		9.893	98.93			
	SPI	1	50	50.88	101.3	0.863	0.86	0.378
		2		49.13	98.81			
		3		50.47	104.7			
		4		49.8	99.87			
		5		49.54	99.46			
		6		48.81	98.12			
DYTOR+	TOR	1	20	20.89	104.4	1.147	1.154	0.811
		2		19.14	95.73			
		3		20.58	102.9			
		4		19.47	97.35			
		5		19.36	96.87			
		6		19.83	99.15			
	SPI	1	50	50.14	100.2	0.86	0.960	0.55
		2		50.48	100.9			
		3		48.91	97.82			
		4		49.45	98.91			
		5		49.81	99.63			
		6		49.31	98.63			

TOR is Torsemide
SPI is Spironolactone

TABLE-4

ASSAY OF COMMERCIAL FORMULATION BY UV-SPECTROSCOPY (ABSORBANCE RATIO METHOD)

Formulation	Drug	S.No.	Amount Labeled (mg/tab)	Amount Estimated (mg/tab)	% Amount found	S.D. (+/-)	R.S.D.	S.E.
TORLACTONE	TOR	1	10	9.833	98.33	0.88	0.88	0.51
		2		10.13	101.3			
		3		10.38	103.8			
		4		9.711	97.11			
		5		9.965	99.65			
		6		10.03	100.3			
	SPI	1	50	50.89	100.8	0.70	0.71	0.40
		2		48.25	98.25			
		3		49.88	98.42			
		4		47.22	97.51			
		5		51.45	101.3			
		6		49.96	98.96			
DYTOR+	TOR	1	20	19.69	98.45	0.69	0.70	0.40
		2		20.31	101.5			
		3		19.17	95.85			
		4		20.38	101.9			
		5		20.83	104.1			
		6		19.35	96.25			
	SPI	1	50	49.69	99.38	1.31	1.32	0.75
		2		51.55	103.1			
		3		47.24	94.48			
		4		49.81	99.62			
		5		49.25	98.55			
		6		50.91	101.8			

TOR is Torsemide
SPI is Spironolactone

TABLE-5

ASSAY OF COMMERCIAL FORMULATION BY UV-SPECTROSCOPY (DERIVATIVE SPECTROSCOPY METHOD)

Formulation	Drug	S.No	Amount Labeled (mg/tab)	Amount Estimated (mg/tab)	% Amount found	S.D. (+/-)	R.S.D.	S.E.
TORLACTONE	TOR	1	10	9.89	98.93	1.7	1.07	0.43
		2		10.16	101.6			
		3		9.62	96.2			
		4		10.43	104.3			
		5		9.83	98.33			
		6		10.18	101.8			
	SPI	1	50	50.36	100.3	0.46	0.46	0.18
		2		50.41	100.4			
		3		49.81	98.12			
		4		48.21	98.72			
		5		48.44	98.74			
		6		51.21	102.7			
DYTOR+	TOR	1	20	20.81	104.5	2.02	2.01	0.43
		2		19.38	96.90			
		3		20.34	101.7			
		4		19.26	96.3			
		5		20.16	100.8			
		6		19.98	99.93			
	SPI	1	50	51.12	102.2	1.42	1.43	0.82
		2		48.41	96.82			
		3		49.21	98.42			
		4		49.91	99.82			
		5		50.63	101.2			
		6		50.41	100.8			

TOR is Torsemide
SPI is Spironolactone

TABLE -6

RECOVERY STUDIES –SIMULTANEOUS EQUATION METHOD

Formulation	Drug	S.No.	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	Amount Recovered (µg/ml)	% Recovery
TORLACTONE	TOR	1	0.8	4	4.78	3.98	99.54
		2	0.8	6	6.91	6.10	101.6
		3	0.8	8	8.85	8.05	100.6
	SPI	1	4	4	7.94	3.94	98.52
		2	4	6	10.5	6.05	100.8
		3	4	8	12.23	8.23	102.9
DYTOR+	TOR	1	1.6	4	5.73	4.13	103.2
		2	1.6	6	7.58	5.98	99.68
		3	1.6	8	9.59	7.99	99.87
	SPI	1	4	4	8.41	4.11	101.2
		2	4	6	9.87	5.87	97.83
		3	4	8	12.1	8.19	102.3

TOR is Torsemide
SPI is Spironolactone

TABLE -7

RECOVERY STUDIES–ABSORBANCE RATIO METHOD

Formulation	Drug	S.No.	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	Amount Recovered (µg/ml)	% Recovery
TORLACTONE	TOR	1	0.8	4	4.96	4.16	104.8
		2	0.8	6	6.71	5.91	98.5
		3	0.8	8	8.98	8.81	102.2
	SPI	1	4	4	7.64	3.64	96.5
		2	4	6	10.21	6.21	103.5
		3	4	8	12.01	8.01	101.2
DYTOR+	TOR	1	1.6	4	5.67	4.07	101.3
		2	1.6	6	7.54	5.94	96.25
		3	1.6	8	9.61	8.01	99.6
	SPI	1	4	4	8.04	4.04	101.1
		2	4	6	10.51	6.51	108.5
		3	4	8	11.39	7.39	92.37

TOR is Torsemide
SPI is Spironolactone

TABLE -8

RECOVERY STUDIES –DERIVATIVE SPECTROSCOPY METHOD

Formulation	Drug	S.No.	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	Amount Recovered (µg/ml)	% Recovery
TORLACTONE	TOR	1	0.8	4	4.85	4.05	101.5
		2	0.8	6	6.91	6.11	101.8
		3	0.8	8	8.91	8.11	101.3
	SPI	1	4	4	7.89	3.89	97.25
		2	4	6	10.22	6.22	103.66
		3	4	8	12.41	8.41	105.12
DYTOR+	TOR	1	1.6	4	5.54	3.94	98.5
		2	1.6	6	7.71	6.11	101.8
		3	1.6	8	9.90	8.3	103.7
	SPI	1	4	4	8.21	4.21	105.2
		2	4	6	9.94	5.94	99.5
		3	4	8	11.89	7.89	98.86

TOR is Torsemide
SPI is Spironolactone

TABLE-9

PRECISION (INTERDAY)

Formulation	Drug	S.No.	Amount Labeled (mg/tab)	Amount Estimated (mg/tab)	% Amount found	S.D (+/-)	R.S.D	S.E
TORLACTONE	TOR	1	10	9.833	98.33	1.33	1.03	0.76
		2		10.13	101.3			
		3		10.38	103.8			
	SPI	1	50	50.89	100.8	0.98	0.99	0.56
		2		48.25	98.25			
		3		49.88	98.42			
DYTOR+	TOR	1	20	19.69	98.45	0.11	0.13	0.06
		2		20.31	101.5			
		3		19.17	95.85			
	SPI	1	50	49.69	99.38	0.24	0.24	0.12
		2		51.55	103.1			
		3		47.24	94.62			

TOR is Torsemide
SPI is Spironolactone

TABLE-10

PRECISION (INTRADAY)

Formulation	Drug	S.No.	Amount Labeled (mg/tab)	Amount Estimated (mg/tab)	% Amount found	S.D (+/-)	R.S.D	S.E
TORLACTONE	TOR	1	10	9.711	97.11	0.67	0.64	0.31
		2		9.96	99.65			
		3		10.03	100.3			
	SPI	1	50	50.89	100.8	0.88	0.83	0.34
		2		48.25	98.25			
		3		49.88	98.96			
DYTOR+	TOR	1	20	19.69	98.54	1.74	1.43	0.58
		2		20.38	101.9			
		3		20.83	104.2			
	SPI	1	50	49.81	99.62	0.20	0.21	0.19
		2		49.25	98.5			
		3		50.91	101.8			

TOR is Torsemide
SPI is Spironolactone

TABLE-11

OPTICAL CHARACTERISTICS OF TORSEMIDE IN 0.1 N HCL

PARAMETERS	TORSEMIDE
λ max	286.5
Beer's law limit(µg/ml)	4-32 µg/ml
Sandell's sensitivity (mg/cm²/0.001A.v)	0.027923211
Molar absorptivity (1/mol/cm)	12866.451
Correlation Co- efficient	0.9998
Slope (m)	0.0355776950
Intercept (c)	0.00199851301
LOD (µg/ml)	0.15775
LOQ (µg/ml)	0.47804

TABLE -12

QUANTIFICATION OF TORSEMIDE IN FORMULATION-1 (TORSINEX)

S.NO.	LABLE CLAIM	AMOUNT OBTAINED	% OBTAINED	AVERAGE	% R.S.D
1	10 mg	9.851	98.51		
2	10 mg	9.795	97.99		
3	10 mg	9.781	98.84	98.79 %	0.1984816
4	10 mg	9.865	98.65		
5	10 mg	9.893	98.93		
6	10 mg	9.937	99.87		

TABLE -13

QUANTIFICATION OF TORSEMIDE IN FORMULATION-2 (HENLETOR)

S.NO.	LABLE CLAIM	AMOUNT OBTAINED	% OBTAINED	AVERAGE	% R.S.D
1	20 mg	19.865	99.328		
2	20 mg	19.647	98.235		
3	20 mg	19.759	98.798	99.123%	0.1565388
4	20 mg	19.731	99.518		
5	20 mg	19.703	98.518		
6	20 mg	19.731	98.65		

Table -14

QUANTIFICATION OF TORSEMIDE IN FORMULATION-3 (TIDE)

S.NO.	LABLE CLAIM	AMOUNT OBTAINED	% OBTAINED	AVERAGE	% R.S.D
1	10 mg	9.950	99.500		
2	10 mg	9.906	99.063		
3	10 mg	9.893	99.938	98.935%	0.08412566
4	10 mg	9.893	98.936		
5	10 mg	9.865	98.657		
6	10 mg	9.851	98.916		

Table-15

QUANTIFICATION OF TORSEMIDE IN FORMULATION-4 (DYTOR)

S.NO.	LABLE CLAIM	AMOUNT OBTAINED	% OBTAINED	AVERAGE	% R.S.D
1	10 mg	9.950	99.500		
2	10 mg	9.925	99.925		
3	10 mg	9.950	99.500	99.488%	0.06371167
4	10 mg	9.913	99.135		
5	10 mg	9.903	99.086		
6	10 mg	9.925	99.253		

Table-16

RECOVERY STUDIES OF TORSEMIDE IN FORMULATION (TORSINEX)

Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Average
10.027	2.5	12.76	2.49	99.6	
9.9219	5.0	14.92	4.99	99.8	101.49
9.5288	7.5	17.45	7.92	104.66	%
10.027	10	19.78	9.76	97.60	

Table-17

RECOVERY STUDIES OF TORSEMIDE IN FORMULATION (HENLETOR)

Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Average
19.73	4	24.06	4.32	108.21	
19.64	8	27.57	7.93	99.12	100.59
19.75	12	31.08	11.32	94.44	%

Table-18

RECOVERY STUDIES OF TORSEMIDE IN FORMULATION (TIDE)

Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Average
19.89	4	24.28	4.39	109.18	
19.85	8	27.22	7.37	95.21	104.34
19.86	12	32.84	12.98	108.62	%

Table-19

RECOVERY STUDIES OF TORSEMIDE IN FORMULATION (DYTOR)

Amount Present (µg/ml)	Amount Added (µg/ml)	Amount estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Average
19.95	4	24.11	4.16	105.41	
19.92	8	27.51	7.59	94.87	101.97
19.95	12	32.60	12.65	105.87	%

TABLE-20

OPTICAL CHARECTERSTICS OF TORSEMIDE AND SPIRONOLACTONE BY RP-HPLC METHOD

PARAMETERS	TORSEMIDE	SPIRONOLACTONE
λ max	262	262
Beer's law limit (µg/ml)	5-25	5-25
Correlation Co- efficient	0.9998	0.9999
Slope (m)	272159.04	187484.83
Intercept (c)	30617.3	-5990.75
LOD (µg/ml)	0.1919119	0.62941843
LOQ (µg/ml)	0.581549668	1.9073285
Standard Error	329160.45	209542.88

TABLE -21

SYSTEM SUITABILITY PARAMETERS FOR THE OPTIMIZED CHROMATOGRAM BY RP – HPLC METHOD

PARAMETERS	TORSEMIDE	SPIRONOLACTONE
Tailing factor	1.30	1.22
Asymmetrical factor	1.44	1.33
Capacity factor	0.82	4.37
Theoretical plate per unit length	36.26	53.54
Resolution	18.02	

TABLE-22

ASSAY OF COMMERCIAL FORMULATION (DYTOR+) BY RP-HPLC METHOD

DRUG	SAMPLE NO.	LABELED AMOUNT (mg/tab)	AMOUNT FOUND (mg/tab)	PERCENTAGE OBTAINED	AVERAGE %	S.D. (+/-)	% R.S.D.
TOR	1	10	10.44	104.4	100.45	0.54	0.51
	2	10	10.26	102.6			
	3	10	10.48	104.8			
	4	10	9.932	99.32			
	5	10	9.442	94.48			
	6	10	9.72	97.2			
SPI	1	50	50.32	100.64	98.96	0.76	0.71
	2	50	49.50	99.01			
	3	50	50.56	101.12			
	4	50	47.86	95.72			
	5	50	45.84	94.68			
	6	50	48.3	96.6			

TOR is Torsemide
SPI is Spironolactone

TABLE – 23

RECOVERY STUDIES OF TORSEMIDE AND SPIRONOLACTONE BY RP-HPLC METHOD

DRUG	% LEVEL	AMOUNT PRESENT (µg/ml)	AMOUNT ADDED (µg/ml)	AMOUNT ESTIMATED (µg/ml)	AMOUNT RECOVERED	% RECOVERY	S.D. (+/-)	% R.S.D.
TOR	150	2.5	5	7.876	2.876	105.01	1.93	1.88
	200	2.5	7.5	11.34	6.34	113.4		
	250	2.5	10	11.19	6.19	89.54		
SPI	100	12.5	2.5	14.94	2.45	99.96	0.43	0.43
	120	12.5	5	17.69	5.19	101.18		
	130	12.5	7.5	19.98	7.48	99.90		

TOR is Torsemide
SPI is Spironolactone

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